

GenCore version 5.1.6
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OM protein - nucleic search, using frame_plus_pn model

Run on September 9, 2003, 15:54:00 ; Search time 0.001 seconds
(without alignments)

755.66 Million cell-updates/sec

Title: us-09-831-455b-6

Perfect score: 1803

Sequence: 1 MSSWSRQPRKSPGGIOPHVS.....RCAGPEAVKGOTLLAVAKSQ 347

Score: 1803

BLOSUM62

Xgapop 10.0 Xgapext 0.1
Ygapop 10.0 Ygapext 0.1
Fgapop 6.0 Fgapext 0.1
Delp 6.0 Dlext 0.1

Searched:

2 seqs, 1089 residues

Total number of hits satisfying chosen parameters: 4

Minimum DB seq length: 0

Post-processing: Minimum Match 0%

Listing first 4 summaries

Command line parameters:
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-OUT=align_6_nuc -MINMATCH=0.1 -DOOPCL=0 -LOOPEXT=0 -UNITS=bits -START=1
-END=1 -MATRIX=BLOSUM62 -TRANS:human40.cdi -LISM=4 -DOCALIGN=200
-THR=SCORE_PCT -THR:MIN=0 -THR:MAX=4 -MOD=LOCAL -OUTTYPE=PO
-NORM=ext -HEAHSIZE=500 -MINLEN=0 -MAXLEN=2000000000 -NCPU=6 -NO_XLPPXY
-NGC_SCORES=0 -LONGLOG -THRESHOLD=1 -XGAPOP=10 -XGAPEXT=0.1 -FGAPOP=6
-FGAPEXT=0.1 -YGAPEXT=0.1 -DELOP=6 -DLEXT=0.1

Database :
1: /home/sdavid/sdavid-tmp/sep03/steadman455/aav87784.geneseqn1990s:
2: /home/sdavid/sdavid-tmp/sep03/steadman455/aav87784.geneseqn1990s:
Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No. Score Query Match Length DB ID Description

No.	Score	Query	Match	Length	DB	ID	Description
1	1107.8	AAY87784	EST clone ES35	686	H		EST clone ES35. H
2	165.4	AAY87784	EST clone ES35	686	H		EST clone ES35. H
3	116.4	AAY87784	EST clone ES35	686	H		EST clone ES35. H
4	98.0002	AAY87784	EST clone ES35	686	H		EST clone ES35. H

ALIGNMENTS

RESULT	1	AAV87784 standard; cDNA; 686 BP.
XX	AC	XX
XX	DT	12-FEB-1999 (first entry)
DE	DE	EST clone ES35.
KW		Expressed sequence tag; secreted protein; haematopoiesis regulator;

tissue growth; activin; inhibin; tumour invasion suppressor; EST; human; KW

chemotaxis; chemokines; haemostasis; gene therapy; thrombolytic; receptor; ligand; anti-inflammatory; tumour inhibitor; ds.

KW
XX
OS
XX

Homo sapiens.

XX
PN
XX

WO9845437-A2.

XX
PA (GEMY) GENETICS INST. INC.
PT Agostino MJ, Jacobs K, Lavallie ER, McCoy JM, Marberg D;
PT Racine LA, Spaulding V, Tracy M;
XX DR WPI; 1999-07478706.

XX
PT New polynucleotides encoding human secreted proteins - derived from ovary, pituitary, retina and colon cDNA libraries
XX PS Claim 1; Page 178; 611pp; English.

CC The present sequence represents an expressed sequence tag (EST), and is a polynucleotide of the invention. The polynucleotides of the invention are all secreted EST sequences isolated from a variety of human tissue sources. The EST sequences and proteins encoded by them are predicted to have useful biological activities which would make them suitable for treating, preventing or ameliorating medical conditions in humans and animals, although no supporting data is given. Suggested activities include nutritional activity, immune stimulating or suppressing activity, haemostatic regulating activity, chemotactic/chemokinetic activity, haemostatic and thrombolytic activity, chemokinetic activity, haemostatic activity, cathepsin/tumour invasion suppressor activity, anti-inflammatory activity, and therapeutic. The EST sequences are also stated to be useful for gene therapy.

CC Sequence 686 BP; 137 A; 243 C; 177 G; 129 T; 0 other;

SQ

Alignment Scores:

Pred. No.: 0
Score: 1107.80

Length: 686
Matches: 218

Percent Similarity: 98.64%

Conservative: 0

Best Local Similarity: 98.64%

Mismatches: 2

Indels: 61.44%

Query Match: 2

Gaps: 0

DB: us-09-831-455b-6 (1-347) x AAV87784 (1-686)

QY 1 MetserserprpserarglnArpprotysarProglylyieglnProHisvaiser 20

Db 27 ATGCCTCTGGAGCACGCCAAAGCCAGGGCATCAACCCATGTTCT 86

ACCESSION:AA622495

QY 21 ArgthrlepheleuleleuleleuaAlaserlatrpglyvalThrleusePro 40

Db 87 AGAACCTCTGGCTGGCTCGTGGCAGCTAACCTGAGCCCC 146

DT 41 LysaspypsglyvalheArgSerAspHisgluSerSerIrescysglnProPro 60

OY 147 AACAACTCCAGGGTCGCTCAGCATGGCAGCTCCATCTCTGTCAACCC 206

AC 61 GluIleProGlyTyrIleProAlaAspThrValIleLeuLavaIgluLphePheAslu 80

XX 207 GAATCCCGGCTACTGGCCAGCGACACGGCAGCTGGCGTGAATCTCACCTG 266

DE 81 ThrIleIleProAlaAsnLeuIleIleGlyAlaSerLysIleGluIleLeuHsleIer 100

XX 267 ACCCACCTGCCAACCTCAGGGCCTTAAGCTCAAGATGACCTC 326

QY 101 SERASGLYLeuGlusSerLeuSerProGluPheLeuArgProValProGlnLeuArgIval 120
 CC ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
 CC include nutritional activity; immune stimulating or suppressing activity;
 CC haemostatic; regulating activity; tissue growth activity; anti-inflammation
 CC activity; inhibitory activity; chemotactic; chomokinetic activity; haemostatic
 CC and thrombolytic activity; receptor/ligand activity; anti-inflammatory
 CC activity; cationin/tumour invasion suppressor activity; tumour inhibition
 CC activity. The EST sequences are also stated to be useful for gene
 therapy.

QY 241 LeuAspThrLeuLeuLysGluAsnGlnLeuGluValLeuGluValAsnTrpIle 160
 DB 447 ACCCGGAGACCCGGTGAATGAACTGGCTCGGCCGAACTTCCTGGGGCAGTCAGCTGGTG 386
 QY 161 HisGlyLeuLysAlaLeuGlyHisLeuAspLeuSerGlyAsnArgLeuGlyLeuPro 180
 DB 572 AGCGCTGAACTGCGGCTGGGTCAGCTGACCTGCTGGAAACCCCTGGAAACTGCC 566
 QY 572 ProGlyLeuLeuAlaAsnPhthrLeuLeuArgThrLeuAspLeuGlyLeuGluGlnIle 200
 DB 567 CCCGGCTGCTGGCAACTCACCCCTGGACCTGGCGCTGGACCTGGAGAACCTG 626
 QY 201 GlutThrLeuProAspLeuLeuArgGlyProLeuGlnLeuGluArgIle 220
 DB 627 GAGACCTTGCACCTGACCTCTGAGGGGCGGCTGGCAATTAGACGCC-CATTCTCGA 685
 QY 220 u 220
 DB 686 G 686

RESULT 2

QY AAV87784/C
 ID AAV87784 standard; cDNA; 686 BP.

AC AAV87784;

KX DT 12-FEB-1999 (first entry)

DE EST clone ES35.

XX

KW Expressed sequence tag; secreted protein; haematopoiesis regulator; tissue growth; activin; inhibit; tumour invasion suppressor; EST; human; chemotaxis; chemokinesis; haemostasis; gene therapy; thrombolysis; receptor; ligand; anti-inflammatory; tumour inhibitor; ds.

KW Homo sapiens.

XX PN WO9845437-A2.

XX PD 15-OCT-1998.

XX PF 10-APR-1998; 9BWO-US06956.

XX PR 10-APR-1997; 9US-0837312.

XX PA (GEMT) GENETICS INST INC.

XX Agostino MJ, Jacobs K, Lavallie ER, McCoy JM, Merberg D; Racine LA, Spaulding V, Treacy M; DR WPI; 1999-070078/06.

PT New Polynucleotides encoding human secreted proteins - derived from e.g. human blood, kidney, foetal lung, placenta, testes, brain, ovary; pituitary, retina and colon cDNA libraries

PS Claim 1; Page 1/8; 641PP; English.

QY The present sequence represents an expressed sequence tag (EST), and is a polynucleotide of the invention. The polynucleotides of the invention are all secreted EST sequences isolated from a variety of human tissue sources. The EST sequences and proteins encoded by them are predicted to have useful biological activities which would make them suitable for treating, preventing or ameliorating medical conditions in humans and

CC animals, although no supporting data is given. Suggested activities include nutritional activity; immune stimulating or suppressing activity; haemostatic; regulating activity; tissue growth activity; anti-inflammation activity; inhibitory activity; chemotactic; chomokinetic activity; haemostatic and thrombolytic activity; receptor/ligand activity; anti-inflammatory activity; cationin/tumour invasion suppressor activity; tumour inhibition therapy.

SQ Sequence 686 BP; 137 A; 243 C; 177 G; 129 T; 0 other;

Alignment Scores:

Pred. No.: 0 Length: 686
 Score: 165.40 Matches: 88
 Percent similarity: 23.71% Conservative: 22
 Best Local Similarity: 18.97% Mismatches: 62
 Query Match: 9.17% Indels: 301
 DB: 2 Gaps: 1 6

us-09-855b-6 (1-347) x AAV87784 (1-666)

QY 27 leuIleLeuAlaSerAlaLysPheLeuSerProIleAspCysGlnValPhe 46
 DB 672 GTCCTATTCAGTG-----GACCCCTAGGGAGTCAGGGCA 634

QY 47 ArgSerAspIleGlySer-----SerIleSerCys----- 56
 DB 633 AGGTCCCAACTGGTTCTCCCCAGGCA--GGTCCCAGGAGGGTGAAGTGGCCAG 576

QY 57 GlnProProIleGlyLeuProGlyIleProAlaSerPhrValHisLeuAlaGlu 76
 DB 575 CAGCCGGGGGCAGTRCCGAGGGTCCCA--GACAG-GGCCAGAT-GCCCAGAG 520

QY 77 PhePheAsnLeuThrHisLeuProAlaAsnLeuLeuGly-----AlaSerIle-S-Le 94
 DB 519 -----CCTTCAGGCGGTGAGCCAGACCT 493

QY 94 uGlnGlnIleuHisIleu---SerSerAsn----- 105
 DB 492 CCAGGACCTCCACSTGTTGTTCAATACCAGGGTGTCCAGGGTGTCTGGCTAGGCTGA 433

QY 105 userleu-----SerPro-----GluPhe-----LeuArgProVal-ProGlnLeuAr 119
 DB 432 AGAGCCCGAGGGCAGGCCGTCAGGGGTTGCGGTAGATCAGCACCTCAGCTGC 373

QY 119 gValLeuAspLeuThrArgAsnAlaLeuThrGlyLeuProProGlyLeuPheGlnAlaSe 139
 DB 372 G----- 372

QY 139 rAlaThrLeuAspThrLeuValLeuLysGluAsnGlnLeuGluValLeuSerIrr 159
 DB 371 -----CACTG 367

QY 159 PheIleGlyLeuLysAlaLeuGlyHisLeuAspLeuSerGlyAsnArgLeuArgIle 179
 DB 366 GCC-GCGGAATTCGGCG----- 349

QY 179 uproProGlyLeuLeuAlaAsnPhthrLeuLeuArgThrLeuAspLeuGlyLysNgl 199
 DB 349 ----- 349

QY 199 nLeuGluThrLeuProProAspLeuLeuArgGly-----ProLeuGluLeuGluArgIle 216
 DB 348 -----AGAGCTTCAGCCATG-----CTGGAGGT 319

QY 217 -----LeuIleLeuGly-----AsnIleu-----GlnValLeuGlyLys 230
 DB 318 GCAATTCCTGGAGCTTGAAGCGCCCTGGAGGAGGTTGCGCAGGT 270

QY 230 PleuLeuProGlnProAspLeuLeuArgIleuLeuAsnGly-----AsnIleu 247
 DB 269 -----GGCAGGTGAGATT 253

	Query Match:	6.468	Indels:	225
	Gaps:	6		
Qy	247 ysLeukLaarg---ValAlaAlaGly-AlaPheGlyLysLeuArgin---LeuAsp 263			
Db	252 CCAAGGCCAGGNGCAGCGTGGCCTGGGAGGAGGAGCCGGGAAATTCGGCAGGGGTGAC 193			
Qy	254 MetLeuAspLeuSerAsnAspSerIleuAspSerIleuAspSerIleuAspSerIleuAsp 193			
Db	192 AGGA-TATGG-AGCAGCCAGGAGTGACGGAAACACTGGGACTTGGGCTCAG- 138			
Qy	284 GLYGINPro-----AsnTrpAspMetArgasp-Glyphe---AspIle 296			
Db	137 SGAGGAGCCCCAGGCGAGGAGCTGCACACGACGAGCAGGAGCTGAGATG 78			
Qy	297 SerGlynn---ProTrpIleCysAspGlnAsnLeuAspAspLeuTyraGtrp---Leu 314			
Db	77 GGTTGAGATGCCCTGGGTTT-----TGGTGCTG 45			
Qy	315 GAAAGA-GlnLySAspIys-----MetPheSerGln 324			
Db	44 CGCCGCCCCAGGAGCATGGTAGGCTCTTGGCGGA 5			
RESULT 3				
LOCUS	AA622495			
DEFINITION	nq5904.s1_NCI_CGAP-C09 Homo sapiens cDNA clone IMAGE:148143 3'			
ACCESSION				
VERSION	AA622495.1			
KEYWORDS	EST.			
SOURCE	Homo sapiens (human)			
ORGANISM	Homo sapiens			
JOURNAL	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;			
COMMENT	Mammalia; Eutheria; Primates; Catarrhini; Hominoidea; Homo.			
REFERENCE	1 (bases 1 to 403) NCI-CGAP http://www.ncbi.nlm.nih.gov/ncicgap.			
AUTHORS	Ph.D.			
TITLE	National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index			
FEATURES	Unpublished			
source	Contact: Robert Straubberg, Ph.D. Email: cgrapbs-r@mail.nih.gov Tissue Procurement: Ilan Kirsch, M.D., Michael R. Emmert-Buck, M.D., CDNA Library Preparation: M. Bento Soares, Ph.D. cDNA Library Arrayed by: Greg Lennon, Ph.D. DNA Sequencing by: Washington University Genome Sequencing Center Clone distribution: NCI-CGAP clone distribution information can be found through the I.M.A.G.E. Consortium/LINN at: www-bio.llnl.gov/dbp/image/Image.html Seq primer: -40m3 fwd. Et from Amersham.			
FEATURES	Location/Oqlifiers			
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organism="Homo sapiens"				
/mol_type="mRNA"				
/clone="IMAGE:148143"				
/tissue_type="colon tumor RER1"				
/lab_host="DH10B"				
/clone_lib="NCI_CGAP-C09"				
note="Organ: colon; Vector: PT7/3D-Pac (Pharmacia) with a modified polylinker; 1st strand cDNA was prepared from RER1 colon tumor, and was then primed with a Not I-RI adaptor (Pharmacia), digested with Not I and cloned vector. Library was not normalized. Library was constructed by Bento Soares and M. Fatima Bonaldo (soares4)."				
BASE COUNT	105 a 89 c 117 g 92 t			
Alignment scores:				
Pred. No.:	0	Length:	403	
Score:	116.40	Matches:	45	
Percent Similarity:	20.69%	Conservative:	21	
Best Local Similarity:	14.11%	Mismatches:	29	
RESULT 4				
LOCUS	AA622495/c			
DEFINITION	nq5904.s1_NCI_CGAP-C09 Homo sapiens cDNA clone IMAGE:148143 3'			
ACCESSION	AA622495			
VERSION	AA622495.1			
KEYWORDS	EST.			
SOURCE	Homo sapiens (human)			
ORGANISM	Homo sapiens			
JOURNAL	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;			
COMMENT	Mammalia; Eutheria; Primates; Catarrhini; Hominoidea; Homo.			
REFERENCE	1 (bases 1 to 403) NCI-CGAP http://www.ncbi.nlm.nih.gov/ncicgap.			
AUTHORS	National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index			
FEATURES	Unpublished			
source	Contact: Robert Straubberg, Ph.D. Email: cgrapbs-r@mail.nih.gov Tissue Procurement: Ilan Kirsch, M.D., Michael R. Emmert-Buck, M.D., CDNA Library Preparation: M. Bento Soares, Ph.D. cDNA Library Arrayed by: Greg Lennon, Ph.D. DNA Sequencing by: Washington University Genome Sequencing Center Clone distribution: NCI-CGAP clone distribution information can be found through the I.M.A.G.E. Consortium/LINN at: www-bio.llnl.gov/dbp/image/Image.html Seq primer: -40m3 fwd. Et from Amersham.			
FEATURES	Location/Oqlifiers			
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/clone="IMAGE:148143"				
/tissue_type="colon tumor RER1"				
/lab_host="DH10B"				
/clone_lib="NCI_CGAP-C09"				
note="Organ: colon; Vector: PT7/3D-Pac (Pharmacia) with a modified polylinker; 1st strand cDNA was prepared from RER1 colon tumor, and was then primed with a Not I-RI adaptor (Pharmacia), digested with Not I and cloned vector. Library was not normalized. Library was constructed by Bento Soares and M. Fatima Bonaldo (soares4)."				
BASE COUNT	105 a 89 c 117 g 92 t			
Alignment scores:				
Pred. No.:	0	Length:	403	
Score:	116.40	Matches:	45	
Percent Similarity:	20.69%	Conservative:	21	
Best Local Similarity:	14.11%	Mismatches:	29	
RESULT 5				
LOCUS	AA622495			
DEFINITION	nq5904.s1_NCI_CGAP-C09 Homo sapiens cDNA clone IMAGE:148143 3'			
ACCESSION	AA622495			
VERSION	AA622495.1			
KEYWORDS	EST.			
SOURCE	Homo sapiens (human)			
ORGANISM	Homo sapiens			
JOURNAL	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;			
COMMENT	Mammalia; Eutheria; Primates; Catarrhini; Hominoidea; Homo.			
REFERENCE	1 (bases 1 to 403) NCI-CGAP http://www.ncbi.nlm.nih.gov/ncicgap.			
AUTHORS	National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index			
FEATURES	Unpublished			
source	Contact: Robert Straubberg, Ph.D. Email: cgrapbs-r@mail.nih.gov Tissue Procurement: Ilan Kirsch, M.D., Michael R. Emmert-Buck, M.D., CDNA Library Preparation: M. Bento Soares, Ph.D. cDNA Library Arrayed by: Greg Lennon, Ph.D. DNA Sequencing by: Washington University Genome Sequencing Center Clone distribution: NCI-CGAP clone distribution information can be found through the I.M.A.G.E. Consortium/LINN at: www-bio.llnl.gov/dbp/image/Image.html Seq primer: -40m3 fwd. Et from Amersham.			
FEATURES	Location/Oqlifiers			
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note="Organ: colon; Vector: PT7/3D-Pac (Pharmacia) with a modified polylinker; 1st strand cDNA was prepared from RER1 colon tumor, and was then primed with a Not I-RI adaptor (Pharmacia), digested with Not I and cloned vector. Library was not normalized. Library was constructed by Bento Soares and M. Fatima Bonaldo (soares4)."				
BASE COUNT	105 a 89 c 117 g 92 t			
Alignment scores:				
Pred. No.:	0	Length:	403	
Score:	116.40	Matches:	45	
Percent Similarity:	20.69%	Conservative:	21	
Best Local Similarity:	14.11%	Mismatches:	29	
RESULT 6				
LOCUS	AA622495			
DEFINITION	nq5904.s1_NCI_CGAP-C09 Homo sapiens cDNA clone IMAGE:148143 3'			
ACCESSION	AA622495			
VERSION	AA622495.1			
KEYWORDS	EST.			
SOURCE	Homo sapiens (human)			
ORGANISM	Homo sapiens			
JOURNAL	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;			
COMMENT	Mammalia; Eutheria; Primates; Catarrhini; Hominoidea; Homo.			
REFERENCE	1 (bases 1 to 403) NCI-CGAP http://www.ncbi.nlm.nih.gov/ncicgap.			
AUTHORS	National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index			
FEATURES	Unpublished			
source	Contact: Robert Straubberg, Ph.D. Email: cgrapbs-r@mail.nih.gov Tissue Procurement: Ilan Kirsch, M.D., Michael R. Emmert-Buck, M.D., CDNA Library Preparation: M. Bento Soares, Ph.D. cDNA Library Arrayed by: Greg Lennon, Ph.D. DNA Sequencing by: Washington University Genome Sequencing Center Clone distribution: NCI-CGAP clone distribution information can be found through the I.M.A.G.E. Consortium/LINN at: www-bio.llnl.gov/dbp/image/Image.html Seq primer: -40m3 fwd. Et from Amersham.			
FEATURES	Location/Oqlifiers			
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/clone="IMAGE:148143"				
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/lab_host="DH10B"				
/clone_lib="NCI_CGAP-C09"				
note="Organ: colon; Vector: PT7/3D-Pac (Pharmacia) with a modified polylinker; 1st strand cDNA was prepared from RER1 colon tumor, and was then primed with a Not I-RI adaptor (Pharmacia), digested with Not I and cloned vector. Library was not normalized. Library was constructed by Bento Soares and M. Fatima Bonaldo (soares4)."				
BASE COUNT	105 a 89 c 117 g 92 t			
Alignment scores:				
Pred. No.:	0	Length:	403	
Score:	116.40	Matches:	45	
Percent Similarity:	20.69%	Conservative:	21	
Best Local Similarity:	14.11%	Mismatches:	29	
RESULT 7				
LOCUS	AA622495			
DEFINITION	nq5904.s1_NCI_CGAP-C09 Homo sapiens cDNA clone IMAGE:148143 3'			
ACCESSION	AA622495			
VERSION	AA622495.1			
KEYWORDS	EST.			
SOURCE	Homo sapiens (human)			
ORGANISM	Homo sapiens			
JOURNAL	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;			
COMMENT	Mammalia; Eutheria; Primates; Catarrhini; Hominoidea; Homo.			
REFERENCE	1 (bases 1 to 403) NCI-CGAP http://www.ncbi.nlm.nih.gov/ncicgap.			
AUTHORS	National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index			
FEATURES	Unpublished			
source	Contact: Robert Straubberg, Ph.D. Email: cgrapbs-r@mail.nih.gov Tissue Procurement: Ilan Kirsch, M.D., Michael R. Emmert-Buck, M.D., CDNA Library Preparation: M. Bento Soares, Ph.D. cDNA Library Arrayed by: Greg Lennon, Ph.D. DNA Sequencing by: Washington University Genome Sequencing Center Clone distribution: NCI-CGAP clone distribution information can be found through the I.M.A.G.E. Consortium/LINN at: www-bio.llnl.gov/dbp/image/Image.html Seq primer: -40m3 fwd. Et from Amersham.			
FEATURES	Location/Oqlifiers			
source	1. .403			
organism="Homo sapiens"				
/mol_type="mRNA"				
/clone="IMAGE:148143"				
/tissue_type="colon tumor RER1"				
/lab_host="DH10B"				
/clone_lib="NCI_CGAP-C09"				
note="Organ: colon; Vector: PT7/3D-Pac (Pharmacia) with a modified polylinker; 1st strand cDNA was prepared from RER1 colon tumor, and was then primed with a Not I-RI adaptor (Pharmacia), digested with Not I and cloned vector. Library was not normalized. Library was constructed by Bento Soares and M. Fatima Bonaldo (soares4)."				
BASE COUNT	105 a 89 c 117 g 92 t			
Alignment scores:				
Pred. No.:	0	Length:	403	
Score:	116.40	Matches:	45	
Percent Similarity:	20.69%	Conservative:	21	
Best Local Similarity:	14.11%	Mismatches:	29	
RESULT 8				
LOCUS	AA622495			
DEFINITION	nq5904.s1_NCI_CGAP-C09 Homo sapiens cDNA clone IMAGE:148143 3'			
ACCESSION	AA622495			
VERSION	AA622495.1			
KEYWORDS	EST.			
SOURCE	Homo sapiens (human)			
ORGANISM	Homo sapiens			
JOURNAL	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;			
COMMENT	Mammalia; Eutheria; Primates; Catarrhini; Hominoidea; Homo.			
REFERENCE	1 (bases 1 to 403) NCI-CGAP http://www.ncbi.nlm.nih.gov/ncicgap.			
AUTHORS	National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index			
FEATURES	Unpublished			
source	Contact: Robert Straubberg, Ph.D. Email: cgrapbs-r@mail.nih.gov Tissue Procurement: Ilan Kirsch, M.D., Michael R. Emmert-Buck, M.D., CDNA Library Preparation: M. Bento Soares, Ph.D. cDNA Library Arrayed by: Greg Lennon, Ph.D. DNA Sequencing by: Washington University Genome Sequencing Center Clone distribution: NCI-CGAP clone distribution information can be found through the I.M.A.G.E. Consortium/LINN at: www-bio.llnl.gov/dbp/image/Image.html Seq primer: -40m3 fwd. Et from Amersham.			
FEATURES	Location/Oqlifiers			
source	1. .403			
organism="Homo sapiens"				
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note="Organ: colon; Vector: PT7/3D-Pac (Pharmacia) with a modified polylinker; 1st strand cDNA was prepared from RER1 colon tumor, and was then primed with a Not I-RI adaptor (Pharmacia), digested with Not I and cloned vector. Library was not normalized. Library was constructed by Bento Soares and M. Fatima Bonaldo (soares4)."				
BASE COUNT	105 a 89 c 117 g 92 t			
Alignment scores:				
Pred. No.:	0	Length:	403	
Score:	116.40	Matches:	45	
Percent Similarity:	20.69%	Conservative:	21	
Best Local Similarity:	14.11%	Mismatches:	29	
RESULT 9				
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ACCESSION	AA622495			
VERSION	AA622495.1			
KEYWORDS	EST.			
SOURCE	Homo sapiens (human)			
ORGANISM	Homo sapiens			
JOURNAL	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;			
COMMENT	Mammalia; Eutheria; Primates; Catarrhini; Hominoidea; Homo.			
REFERENCE	1 (bases 1 to 403) NCI-CGAP http://www.ncbi.nlm.nih.gov/ncicgap.			
AUTHORS	National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index			
FEATURES	Unpublished			
source	Contact: Robert Straubberg, Ph.D. Email: cgrapbs-r@mail.nih.gov Tissue Procurement: Ilan Kirsch, M.D., Michael R. Emmert-Buck, M.D., CDNA Library Preparation: M. Bento Soares, Ph.D. cDNA Library Arrayed by: Greg Lennon, Ph.D. DNA Sequencing by: Washington University Genome Sequencing Center Clone distribution: NCI-CGAP clone distribution information can be found through the I.M.A.G.E. Consortium/LINN at: www-bio.llnl.gov/dbp/image/Image.html Seq primer: -40m3 fwd. Et from Amersham.			
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OM protein - protein search, using sw model

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Listing first 45 summaries

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Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

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ALIGNMENTS

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Inhibition of phospholipase A₂

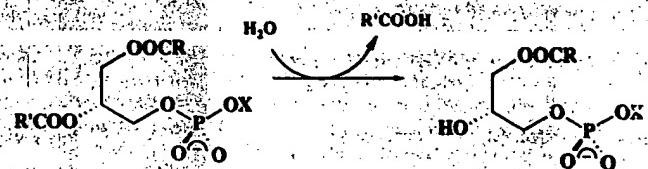
MICHAEL H. GELE,^{*†} MAHENDRA K. JAIN,[†] AND OTTO G. BERG[†]

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ABSTRACT Phospholipases A₂ are involved in inflammatory processes such as the liberation of free arachidonic acid from the membrane pool for the biosynthesis of eicosanoids. Inhibitors of these enzymes are proving useful in determining the biological roles of phospholipases A₂ in complex cellular processes and may also have therapeutic potential. Inhibition of these lipolytic enzymes is more difficult to characterize as the enzymatic reaction occurs at a lipid/water interface. This review focuses on the description of a number of classes of rationally designed phospholipase A₂ inhibitors. The development of a theoretical framework for the proper analysis of inhibitors is presented. Structural studies of phospholipase A₂-inhibitor complexes suggest how the lipolysis reaction is catalyzed. Finally, some recent results on the use of phospholipase A₂ inhibitors in living cells and tissues are revealed.—Gelb, M. H., Jain, M. K., Berg, O. G. Inhibition of phospholipase A₂. *FASEB J.* 8: 916-924; 1994.

Key Words: lipases • inhibitors • arachidonic acid • eicosanoids • protein-lipid interactions

PHOSPHOLIPASES A₂ (PLA₂s)² CATALYZE THE hydrolysis of the ester linkage at the *sn*-2 position of phospholipids to produce free fatty acids and lysophospholipids (Scheme I) (1).



Scheme I

The secreted enzymes (sPLA₂s) from pancreas (2), venoms (3), and inflammatory cells and exudates (4, 5) are the best characterized. These enzymes are small (molecular mass near 14 kDa), require millimolar amounts of Ca²⁺ as a catalytic cofactor but not for binding of the enzyme to membranes (6), and are related both in terms of primary (7) and three-dimensional structure (8-15). The sPLA₂s display almost no preference for different types of naturally occurring phospholipid head groups (substituent X in Scheme I) or the structure of the fatty acyl chains attached to the glycerol backbone (16).

Recently, a high molecular mass (87 kDa) cytosolic phospholipase A₂ (cPLA₂) has been found in human, bovine, and rabbit platelets (17-19), rodent macrophage cell lines (20, 21, 22), human monocytic cell lines (U937 and THP1) (23-26), and rat kidney (27). In contrast to the sPLA₂s, these cPLA₂s are activated by submicromolar amounts of Ca²⁺ and

preferentially hydrolyze phospholipids that have arachidonic acid at the *sn*-2 position. The cPLA₂ is found in the cytosol of cells in the absence of Ca²⁺, and it translocates to the particulate fraction in the presence of submicromolar amounts of Ca²⁺ (23, 24, 26-30). Calcium also promotes the binding of the enzyme to phospholipid vesicles *in vitro* (31, 32). Isolation of the cDNA encoding the cPLA₂ from U937 cells indicates that the enzyme has no regions that are homologous to the sequences of sPLA₂s (23, 33). Furthermore, the amino acid sequence reveals a stretch of 45 residues in the amino-terminal region that shows homology to Ca²⁺-dependent forms of protein kinase C and other Ca²⁺-dependent membrane-binding proteins. A 140 amino acid fragment of this enzyme that contains the amino terminus was shown to bind to membranes in the presence of submicromolar amounts of Ca²⁺ (23).

Gross (34) and co-workers have identified a novel Ca²⁺-independent PLA₂ found in myocardial cytosol. This enzyme prefers plasmalogens that contain arachidonic acid at the *sn*-2 position. Bovine brain cytosol also contains Ca²⁺-independent PLA₂ (35).

Undoubtedly other PLA₂s in cells are involved in phospholipid remodeling and catabolism and in the degradation of phospholipids with oxidatively damaged fatty acyl chains (36).

The sPLA₂ found in inflammatory cells and exudates is almost certainly involved in inflammatory processes (see, for example, refs 37-42). This enzyme is often called the non-pancreatic secreted sPLA₂ because its amino acid sequence is more closely related to those of the sPLA₂s in the venoms from crotalids and viperids (type II enzymes) rather than to those from pancreas (type I enzymes) (7). This enzyme is secreted from activated platelets, mast cells, and other cell types in response to agonists. The role of this enzyme in the degradation of foreign bacteria appears certain (39), its role in mast cell histamine release and T cell activation is being clarified (43, 44), and its involvement in liberating arachidonic acid from the membrane in, for example, a macrophage-derived cell line and endothelial cells has recently been suggested (45, 46). Because arachidonate metabolites are potent mediators of allergic and inflammatory reactions, PLA₂ inhibitors are expected to have therapeutic value. The pancreatic sPLA₂, also called a type I sPLA₂, is found in tissues other than those of digestive function and appears to have a proinflammatory role (47). This enzyme is thought to bind to cells via a specific receptor lead-

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²Abbreviations: PLA₂, phospholipase A₂; cPLA₂, 87-kDa calcium-dependent cytosolic PLA₂ found in mammalian cells; sPLA₂, 14-kDa secreted calcium-dependent PLA₂ found in venoms, digestive fluids, and mammalian inflammatory exudates and cells.

ing to the induction of cyclooxygenase and the subsequent rise in levels of prostaglandins. Evidence is mounting to suggest that the cPLA₂ functions in a signal-transduction pathway leading to the rapid release of arachidonic acid from membranes for the biosynthesis of eicosanoids (48–50). The Ca²⁺-independent PLA₂ may be involved in the degradation of membranes that accompanies myocardial infarction (34) and in the liberation of free arachidonate in hormonally-stimulated aortic smooth muscle cells (51). For all of these reasons, most major pharmaceutical companies have ongoing programs aimed at discovering new inhibitors of PLA₂s.

The analysis of PLA₂ inhibition is more difficult than for most enzymes because the enzymatic hydrolysis of naturally occurring, long-chain phospholipids necessarily occurs at the lipid/water interface. The present article focuses on the design and analysis of tight-binding inhibitors of PLA₂s that have been carried out in the authors' laboratories. The rationale for the design of the inhibitors is discussed because it relates to the catalytic mechanism of PLA₂; this is revealed by X-ray diffraction analysis of PLA₂-inhibitor complexes. The problem of defining the mode of action of inhibitors of interfacial catalysis by PLA₂s is also discussed, as is the development of experimental techniques for analyzing the inhibition. Finally, initial results of PLA₂ inhibition studies with living cells and organs are discussed as they bear on the biological functions of PLA₂s.

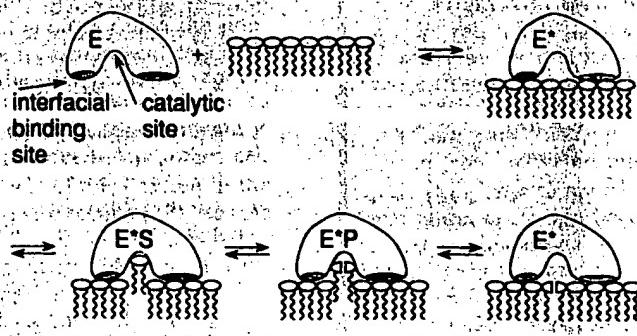
INTERFACIAL CATALYSIS BY PLA₂: THE KINETIC PARADIGM

Before discussing PLA₂ inhibition, it is necessary to briefly describe some basic features of interfacial catalysis by this enzyme. As naturally occurring phospholipids are essentially

insoluble in water and thus form aggregates, PLA₂s must be able to bind to such aggregates in order to gain access to substrate. Two modes of interfacial catalysis can be envisioned, and are illustrated in Fig. 1. In mode 1, it is imagined that the enzyme has a surface that can anchor it to a phospholipid aggregate such as a bilayer and that the enzyme also contains a topologically distinct active site where the lipolysis reaction takes place. In this mode, the enzyme is able to bind to the bilayer without a phospholipid molecule bound in its active site; the active site binding occurs after the enzyme binds to the interface. Thus, the following enzyme species occur in mode 1: E is the enzyme in the aqueous phase, E* is the enzyme bound to the interface but with its active site devoid of substrate and filled with solvent, E*S is the enzyme bound to the interface and with its active site occupied by a single substrate molecule (E*S is analogous to the well-known Michaelis complex, ES, for an enzyme operating in a homogeneous, aqueous-phase environment), and E*P is the enzyme bound to the interface with lipolysis products (fatty acid and lysophospholipid) in its active site. Interfacial catalysis may not necessarily proceed according to mode 1. As shown in Fig. 1, mode 2 can be imagined in which the binding of the enzyme to the interface and the loading of its active site with a molecule of substrate are the same step. In this case, the enzyme species E* does not exist.

As will become clear throughout this review, the distinction between these modes is critical for a proper understanding of most aspects of interfacial catalysis including inhibition. The distinction between these modes comes from considering the possibility of processive interfacial catalysis. In the present context, processivity means that the enzyme can remain bound to a phospholipid vesicle during the course of more than one lipolysis turnover cycle. The most extreme type of processivity is when the enzyme hydrolyzes all the substrate in the outer layer of a phospholipid vesicle without ever leaving the surface. This has been called interfacial catalysis in the "scouting mode" (52) and is schematically illustrated in Fig. 2. Scouting mode catalysis has been observed for sPLA₂s from a variety of sources (venoms, pancreatic juices, and human synovial fluid) (52–54) and for the cPLA₂ from human U937 cells (55). The fact that processive catalysis occurs rules out mode 2 from further consideration. This is because in mode 2, the enzyme necessarily lets go of the interface when the lipolysis products are released from the active site, and the enzyme has no "memory" to enable it to return to the same phospholipid vesicle from which it was previously bound. On the other hand, in mode 1 catalysis the binding of the enzyme to the interface ($E \rightarrow E^*$) is independent of the binding of substrate in the active site ($E^* + S \rightarrow E*S$). If the $E \rightarrow E^*$ step is sufficiently favorable so that the residency time of the enzyme on the interface is longer than the time it takes to complete a lipolysis reaction, processive catalysis will occur. Spectroscopic and chromatographic techniques have shown that sPLA₂s bind to phospholipid vesicles and phospholipid-containing detergent micelles (2, 56–58), but the fact that this binding is independent of the binding of a substrate molecule to the active site comes only from the demonstration that the reaction can be processive (52). Also, pig pancreatic sPLA₂, containing an active site-directed alkylating agent (*p*-bromo-phenacyl group attached to the catalytic histidine residue), is still able to bind to phospholipid aggregates even though substrate cannot bind to the active site because of steric clash with the inactivator (59). To the extent that this property of the inactivated enzyme applies to the native enzyme, this result is further evidence for the mode 1 nature of PLA₂-catalyzed reactions.

Mode 1: Binding of E to the interface and binding of S to the catalytic site are distinct steps



Mode 2: Binding of E to the interface and binding of S to the catalytic site are the same step

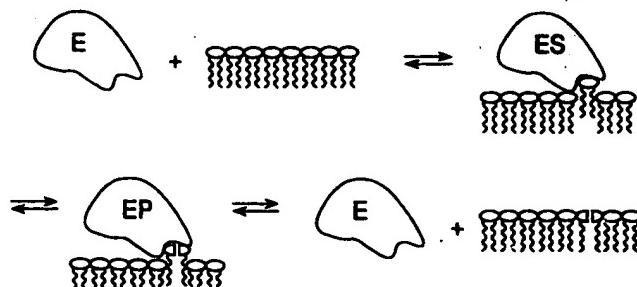


Figure 1. Two possible modes of interfacial catalysis by PLA₂s.

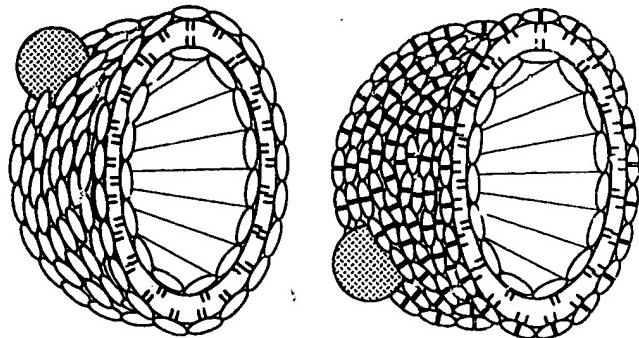


Figure 2. Schematic illustration of the PLA₂-catalyzed hydrolysis of vesicles in the highly processive scooting mode. The enzyme (small sphere) remains irreversibly bound to the vesicle, and only the phospholipids in the outer leaflet of the bilayer are hydrolyzed (the vesicle is sliced in half to reveal the inner leaflet phospholipids, which are not hydrolyzed).

Direct binding studies show that sPLA₂s bind several orders of magnitude tighter to vesicles that are anionic (such as vesicles of phosphatidylmethanol or mixed-lipid vesicles of phosphatidylcholine with phosphatidic acid or phosphatidylserine) compared with vesicles that are charge neutral (such as vesicles composed only of phosphatidylcholine) (16, 52, 54, 57, 60). The enzyme hydrolyzes these anionic vesicles in the scooting mode. With vesicles composed only of phosphatidylcholine, sPLA₂s catalyze interfacial reactions in a less processive mode in which the enzyme hops from one vesicle to another (57). In this case, the reaction progress curves are complex. The processivity and reaction velocity increase as the reaction progresses because accumulation of the fatty acid reaction product gives the vesicles a net negative charge that promotes enzyme binding (61). Kinetic modeling studies suggest that the complex reaction progress curve for the action of snake venom and pancreatic sPLA₂s on large unilamellar phosphatidylcholine vesicles results from dimerization of the enzyme bound to vesicles, and that this dimer persists as the enzyme hops from one vesicle to another (62). Enzyme dimerization, if it occurs, is not obligatory for interfacial catalysis as it has been conclusively shown that monomeric sPLA₂ is fully active on anionic vesicles in the scooting mode, and the enzyme does not aggregate even when multiple enzymes are present on each vesicle (53). cPLA₂s also undergo scooting mode catalysis on vesicles of phosphatidylcholine containing 5–10 mol % of reaction products (1:1 mixture of lysophospholipid and fatty acid) (55). Numerous straightforward experimental protocols have been developed to analyze for scooting behavior (55, 63), and these are not discussed in this review.

Analysis of PLA₂s in the scooting mode provides the most straightforward method for proper analysis of the kinetics of interfacial catalysis. For enzymes that operate in the aqueous phase, it is assumed in all kinetic treatments that each enzyme in the mixture "sees" the same global concentrations of substrates and products. This is not necessarily the case for interfacial enzymes. Consider interfacial catalysis under conditions in which the processivity is low. At some point, a PLA₂ may leave a vesicle and bind to a new one that has not yet had a bound enzyme. This enzyme is effectively at time zero because it encounters only substrate and not products. In another instance, an enzyme may land on a vesicle that is mostly hydrolyzed; this enzyme is effectively at a long reaction time because it experiences product inhibition and substrate depletion. Thus, low processive catalysis leads to a

scrambling of time, making kinetic analyses difficult, if not impossible, to interpret. This problem is eliminated by studying PLA₂s in the scooting mode. Consider the situation in which the enzymes are irreversibly bound to the vesicles and there is sufficient excess of vesicles over enzymes so that vesicles contain at most one bound enzyme. Now each enzyme-containing vesicle will be synchronized in time; at time zero the enzyme sees a full complement of substrate and at the end of the reaction the substrate in the vesicles has been depleted. Thus, the total product formed at any time is simply the amount of product formed in an enzyme-containing vesicle multiplied by the number of such vesicles.

The scooting mode analysis gives, at first glance, an artificial impression of interfacial catalysis, as under physiological conditions the PLA₂ may or may not be operating in a highly processive mode. However, for the reasons given above, the scooting mode analysis allows the kinetic and equilibrium constants that describe the interfacial catalysis to be measured, and these constants will apply to the action of the enzyme in the interface even if the enzyme is coming on and off the membrane. Under the conditions of low processivity, the interfacial kinetic and equilibrium parameters will be difficult to obtain as they are convoluted with the equilibrium constants that describe the binding of the enzyme to the interface; the latter can be measured directly by spectroscopic techniques (63). The problem is further complicated by the fact that the affinity of PLA₂s for the interface depends on the composition of the interface and thus on the fraction of substrate that has been converted to products. All these points have been discussed in detail elsewhere (57, 63, 64).

THE STRUCTURAL BASIS FOR SCOOTING MODE BEHAVIOR OF PLA₂

As stated in the introduction, X-ray structures are available for several sPLA₂s and, in some cases, with short-chain phospholipid analogs bound in their active sites. The structures reveal that the active site consists of a slot that traverses the enzyme into which a single phospholipid molecule can bind and interact with the catalytic residues (Fig. 3). This slot is clearly distinct from the surface of the enzyme that is thought to contact the bilayer (60). These structures and the kinetic results that demonstrate that the E → E^{*} step is distinct from the E^{*} + S → E^{*}S step (52, 60) suggest that a phospholipid molecule must diffuse about 15 Å from the plane of the bilayer into the enzyme bound to the interface in order to reach the catalytic machinery.

These high-resolution structures have also provided a wealth of information about the way in which the enzyme utilizes a histidine residue and a Ca²⁺ ion to help bind the substrate in the active site and to stabilize the substrate-derived transition state (8, 10).

TRUE COMPETITIVE INHIBITORS OF INTERFACIAL CATALYSIS BY PLA₂

The scooting mode analysis is also important for the proper analysis of reversible-binding, competitive inhibitors of PLA₂. History has shown that it is problematic to study reversible inhibitors of PLA₂ under conditions in which the enzyme is weakly bound to the interface (low processivity). This is because it is difficult to sort out whether an inhibitor is working by competing with the substrate for binding to the active site (E^{*} + S → E^{*}S) or by altering the physical nature

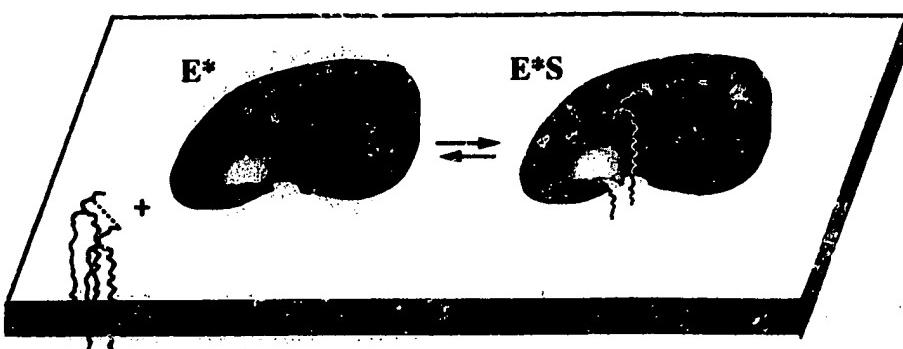


Figure 3. Movement of the substrate from the plane of the bilayer into the catalytic slot of the vesicle-bound sPLA₂. In the bottom-left corner are two phospholipids that interact via their polar head groups (dashed line). The polar head group of the phospholipid in the catalytic slot of the sPLA₂ is shown interacting with an amino acid side chain (dashed line); such an interaction occurs with the cobra venom sPLA₂ but not with the enzymes from pancreas, human synovial fluid, and bee venom.

of the interface so that more of the enzyme desorbs from the surface ($E^* \rightarrow E$). Many previously reported PLA₂ inhibitors have been shown to partition into vesicles and to decrease the fraction of enzyme bound to vesicles (65–67). The characteristics of such nonspecific and therefore uninteresting PLA₂ inhibitors are as follows: 1) inhibition is seen when the enzyme is weakly bound to vesicles but not when the enzyme is tightly bound to vesicles (scouting mode); 2) stereoisomers of inhibitors with chiral centers display similar inhibition potencies; 3) large amounts of inhibitors (corresponding to more than a few mole % inhibitor in the vesicle) are needed to produce the inhibition. The last point is often overlooked. Many PLA₂ assays are carried out radiometrically with small amounts of phospholipid substrate (micromolar), and thus even nanomolar amounts of inhibitor can have a profound effect on the physical nature of the vesicle surface especially for inhibitors that partition favorably into vesicles [contrast this with the situation of enzymes that operate in the aqueous phase where the inhibitor and substrate do not form a coaggregate].

All these problems are avoided by analyzing putative PLA₂ inhibitors in the scouting mode. The presence of additives in anionic vesicles up to 10–20 mol % do not affect the kinetics of interfacial catalysis as the enzyme remains tightly bound to the interface (65). Instead, agents that bind specifically to the active site of the enzyme, the true competitive inhibitors of interfacial catalysis, are detected. The scouting mode analysis has been useful for conducting large-scale screens of compound banks for PLA₂ inhibitors as is routinely done at many pharmaceutical companies (68). Such an analysis does not detect false positive lead compounds that are often encountered in inhibitor screens. By using an assay in which the enzyme is tightly bound to the vesicle, possible inhibitors that bind specifically and tightly to the enzyme in the aqueous phase and prevent the enzyme from binding to the interface may be missed; this is a perfectly valid and useful mode of PLA₂ inhibition. However, the molecular surface of the PLA₂ that contacts the interface is large, and the membrane binding involves the interaction of several amino acids with the interface; thus, it is unlikely that an inhibitor will be able to mask a fraction of this surface sufficient to prevent the $E \rightarrow E^*$ step. Inhibitors that bind in the active site of the enzyme in solution but do not prevent the $E \rightarrow E^*$ step can also be imagined, but if such compounds are driven off the enzyme concomitant with the $E \rightarrow E^*$ step, the inhibitor will be useless. In other words,

one is forced to search for inhibitors that may or may not bind to E but must bind to E^* . Thus, there is little merit in using inhibitor screening strategies that employ E acting on a water-soluble substrate. Other aspects of these arguments have been discussed previously (69).

The equation that describes the inhibition (Eq. 1) is a straightforward extension of the standard steady-state equation that describes competitive inhibition of enzymes that operate in aqueous solution:

$$\frac{v_0}{v_0'} = 1' = \left[(1 + 1/K_1') / (1 + 1/K_M') \right] [X]/(1 - X) \quad (1)$$

Here, v_0 and v_0' are the initial reaction velocities for the action of the enzyme in the scouting mode in the absence or presence of a competitive inhibitor, respectively. K_M' is the interfacial Michaelis constant [just as K_M is the concentration (mol/l) of substrate in the aqueous phase that gives a reaction velocity of $V_{max}/2$, K_M' is the mole fraction of substrate in the vesicle interface that gives $V_{max}/2$]. K_1' is the interfacial dissociation constant for the E^*I complex (the mole fraction of I in the interface that gives $E^*/E^*I = 1$). Thus, the degree of inhibition depends on the affinity of the inhibitor and substrate to the enzyme (K_1' and K_M') and the mole fraction of inhibition in the vesicle (X). [If the amount of inhibitor in the interface is significantly less than the amount of substrate in the vesicle, as is the case for tight-binding inhibitors, the $(1-X)$ term in Eq. 1 can be ignored]. True competitive inhibitors of PLA₂ lead to kinetics that are consistent with Eq. 1 (65, 66).

Phospholipids dispersed in detergent micelles have also been used as PLA₂ substrates and in the analysis of inhibitors (see, for example, refs 70–73). In these systems, tight-binding competitive inhibitors are revealed, and the degree of inhibition depends on the stereochemistry of chiral inhibitors, as is the case with vesicle assays. Some differences between mixed micelles and vesicle assays of inhibition may be noted. The binding of sPLA₂ to mixed micelles is typically of lower affinity than to anionic vesicles, and thus the mole fraction of inhibitor in mixed micelles should be kept low so that one avoids the problem of a shift in the E to E^* equilibrium as previously discussed. It is becoming apparent that reaction velocities for sPLA₂-catalysis lipolysis of mixed micelles is limited by the rate of substrate replenishment in enzyme-containing aggregates (74, 75). This stems from the

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fact that mixed micelles, in contrast to vesicles, contain only a few substrate molecules, and thus significant substrate depletion occurs on the millisecond time scale. Interaggregate exchange of enzyme and/or phosphatidyl lipid is required to maintain the reaction in progress. Thus, it is possible to imagine an inhibitor that functions not by binding directly to E^* but by slowing the rate of interaggregate exchange of substrate and/or enzyme. It may or may not be a valid assumption that such a concern will be avoided if the mole fraction of inhibitor in the aggregate is kept low. Finally, the process or rate-limiting substrate replenishment may distort the value of the mole fraction of inhibitor needed to cause 50% inhibition. This is because the local concentration of substrate in enzyme-containing mixed micelles will be lower than that in the bulk of the micelles that do not have bound enzyme. Thus, the mole fraction of inhibitor needed to cause a 50% reduction will be lower than that predicted by the true K_I^* value, because the inhibitor is competing with an artificially low amount of substrate present in enzyme-containing mixed micelles. This inequality is not possible if the rate of substrate replenishment is fast on the lipolysis turnover time scale so that the local and global steady-state substrate concentrations are equivalent. This situation is not a serious problem as values of K_I^* determined kinetically and under equilibrium conditions are correlated as expected (76).

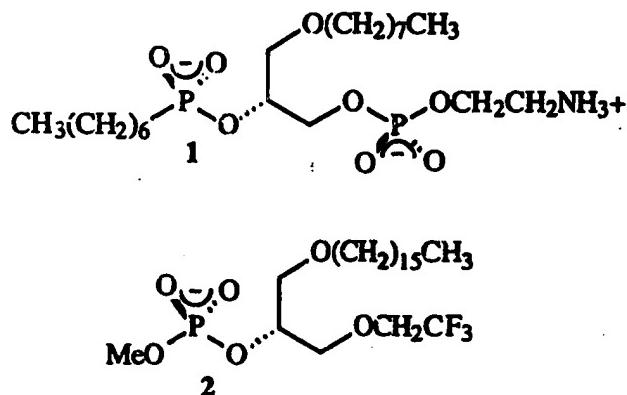
NEUTRAL DILUENTS

Because binding of the enzyme to the interface is independent of the binding of a molecule in the interface to the active site of the enzyme, it should be possible to find an amphiphile that forms an aggregate to which PLA₂ can bind but has no affinity for the active site of the enzyme and so E* has its active site filled with water. Such a compound has been termed a neutral diluent because it can be added to a vesicle of substrate phospholipid and thereby dilute the surface concentration of substrate that the bound enzyme "sees," and at the same time the neutral diluent does not inhibit the enzyme by binding to its catalytic site. The amphiphiles 2-hexadecyl-sn-glycero-3-phosphocholine and hexadecylphosphocholine are neutral diluents for pig pancreatic and human synovial sPLA₂s, respectively (54, 65). This has been established by the demonstration that active site-directed alkylating agents (*p*-substituted phenacyl bromides) modify the catalytic histidine residue of the enzyme in the aqueous phase or bound to an aggregate of neutral diluent at the same rate, which shows that the active site is not protected from alkylation by the binding of the amphiphile. The use of a neutral diluent in combination with an active site-directed alkylating agent provides an independent measure of K_1^* values for PLA₂ inhibitors. This is because when a competitive inhibitor of PLA₂ is present in an aggregate of neutral diluent at mole fraction = K_1^* , half of the bound enzyme will be in the E*I form and the half-time for alkylation of the enzyme will be twice its value obtained in the absence of the inhibitor (65).

STRUCTURAL CLASSES OF REVERSIBLE, TIGHT-BINDING COMPETITIVE INHIBITORS OF PLA₂

Phospholipid analogs that have a phosphonate or phosphate in place of the ester at the *sn*-2 position of phospholipids

(compounds 1 and 2 (below) are tight-binding inhibitors of sPLA₂s but not cPLA₂: (54, 77-79). These substrate analogs are patterned after the putative tetrahedral intermediate that



forms when a water molecule attacks the substrate *sn*-2 ester; they bind to E* about three orders of magnitude tighter than substrates. The X-ray structures of sPLA₂s containing bound compound 1 suggest how the lipolysis reaction is catalyzed (8, 15) (Fig. 4). One nonbridging oxygen of the phosphonate is hydrogen bonded to the protonated imidazole ring of the active site histidine, and the other is directly liganded to the calcium ion. This suggests that the mechanism of substrate hydrolysis involves the attack of a water molecule onto the ester carbonyl carbon assisted by general base catalysis by histidine. The oxyanion, which is derived from the carbonyl oxygen, is stabilized by binding to calcium. The calcium also helps to anchor the substrate in the

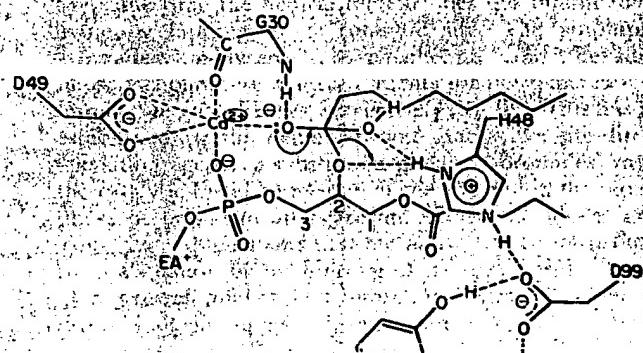


Figure 4. The structural details of the interaction of the putative tetrahedral intermediate with the catalytic residues of sPLA₂s as revealed by X-ray diffraction. The oxyanion of the tetrahedral intermediate interacts with calcium and the amide NH of glycine-30 (pancreatic sPLA₂ residue numbering). The hydroxyl group of the tetrahedral intermediate is hydrogen bonded to the protonated histidine; this amino acid likely protonates the *sn*-2-oxygen as the lysophospholipid is formed. The histidine is also hydrogen bonded to aspartate-99, which receives a hydrogen bond from a tyrosine residue. The *sn*-3-phosphate is liganded to the calcium; the latter is held to the enzyme by aspartate-49 and glycine-30, as shown, as well as two additional carbonyl oxygen ligands from the peptide backbone.

active site by its interaction with one of the nonbridging oxygens of the *sn*-3 phosphate; the other nonbridging oxygen of this phosphate is hydrogen bonded to either a tyrosine (pancreatic and cobra venom sPLA₂s), a threonine (bee venom sPLA₂), or a lysine (human synovial fluid sPLA).

Compound 2 is a member of a novel family of phospholipid analogs that are tight-binding inhibitors of sPLA₂s (79). These compounds have an *sn*-2 phosphate but lack one at the *sn*-3 position. Apparently the polar head group of a phospholipid is not a critical element for its interaction with the active site of sPLA₂s even though it forms interactions with the active site as described in the previous section. This is also apparent from the following additional inhibitor studies. Analogs of phospholipids in which the *sn*-2 ester is replaced by an amide (-CONH-) are tight-binding inhibitors of sPLA₂s (70, 71, 80). The amide hydrogen is hydrogen bonded to the nonprotonated imidazole ring of the active site histidine, and the *sn*-3 phosphate is liganded to the calcium (10, 72). Surprisingly, simple fatty acid amides (RCONH₂) that lack the entire glycerol backbone bind to sPLA₂s with affinities approaching those of phospholipid amide analogues (discussed below) (68).

None of the sPLA₂ inhibitors described cause detectable inhibition of cPLA₂. However, an analog of arachidonic acid in which the COOH group is replaced with a trifluoromethyl ketone (COCF₃) is a tight-binding, reversible inhibitor of cPLA₂; this compound (AACOCF₃) is more than 1,000-fold less potent as an inhibitor of sPLA₂s (73). NMR studies suggest that AACOCF₃ forms a hemiketal adduct with an active site nucleophile [Enz-X-C(OH)(CF₃)-AA] (81). Such an adduct may resemble the tetrahedral intermediate in route to a putative acyl enzyme, but further studies will be required to fully understand the mechanism of catalysis by cPLA₂. Analogs of AACOCF₃ in which the COCF₃ group is replaced by COCH₃, CH(OH)CF₃, or CONH₂ are not cPLA₂ inhibitors (73). A possible reason for the ability of inhibitors that lack a phospholipid-like polar head group to bind tightly to sPLA₂ and cPLA₂ is discussed in the next section.

DIFFERENTIAL INTERACTIONS OF INHIBITORS WITH THE PHOSPHOLIPID INTERFACE AS A CONTROLLING FACTOR FOR POTENCIES OF PLA₂ INHIBITORS

In considering the factors that determine the magnitude of enzyme-inhibitor dissociation constants, one must examine not only the interactions that the inhibitor engages in with the enzyme but also the interactions that the inhibitor forms with its environment when it is not bound to the enzyme. For enzymes in the aqueous phase, experimental and theoretical studies have shown that differential solvation of inhibitors can dictate their relative affinities for enzymes (see, for example, ref 82). Thus, compound A may be a better inhibitor than B not because it forms stronger interactions with the enzyme than B but because as a free species it is less solvated than free B. The same is true for inhibitors of PLA₂, except that the free inhibitor is now in a lipid vesicle instead of in aqueous solution. Thus, differential interactions of inhibitors with adjacent phospholipid molecule in the vesicle can control, in part, the relative affinities of inhibitors for PLA₂ bound to the vesicle surface.

A systematic study was carried out on the effect of varying the structure of the polar head group of *sn*-2 phosphonate-containing phospholipid analogs on the binding to sPLA₂s

(83). The inhibitor potencies were obtained according to Eq. 1 and for the extent of a number of sPLA₂s in vesicles of 1,2-dimyristoyl-*sn*-glycero-3-phosphomethanol in the scooting mode. When comparing inhibitors of PLA₂, it is important to determine whether all of the inhibitors are fully partitioned into the vesicle, otherwise the differences in inhibitor potencies may reflect differences in surface concentrations of inhibitor due to differential partitioning rather than differences in intrinsic affinities of the inhibitors for the enzyme (K_1^*). The scooting mode analysis provides a simple way to assess inhibitor partitioning. The degree of inhibition by a fixed mole fraction of inhibitor in the vesicle is determined at different total reaction volumes. Because the enzyme is tightly bound to the vesicles, the reaction velocity in the absence of inhibitor is independent of the reaction volume. If an inhibitor is present and if it is only partially partitioned into the vesicle phase, the degree of inhibition will decrease as the reaction volume is increased because the latter change will lead to a decrease in the mole fraction of inhibitor in the interface as more and more of it partitions into the aqueous phase. By this criterion, it has been shown that the differences in inhibitor potencies reflect differences in their values of K_1^* (83). A more direct method for determining inhibitor/vesicle partition coefficients has also been reported (84).

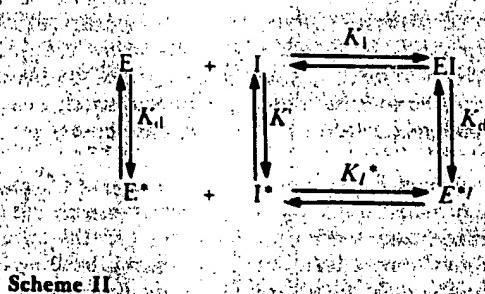
An interesting trend was discovered from this analysis. In the case of the sPLA₂s from porcine and bovine pancreas, bee venom, and human synovial fluid, replacement of the ammonium of the polar head group compound with hydrogen increases the potency by 5- to 10-fold. In contrast, such a change produces no effect on the K_1^* measured with the cobra venom sPLA₂ (83). X-ray structural studies of sPLA₂ inhibitor complexes show that for the enzymes from pancreas, bee venom, and human synovial fluid, the ammonium of the phosphoethanolamine polar head group of the bound phospholipid analog does not directly contact the enzyme. Thus, the increase in inhibitor potency when the ammonium is removed must be because the ammonium group of the free inhibitor forms favorable interactions with the head group phosphate of an adjacent phospholipid in the vesicle, such an interaction is not possible with the analog lacking the ammonium group (Fig. 3). By analyzing the relative binding energies of a series of inhibitors with modified polar head groups to certain sPLA₂s, it is possible to determine the relative energetics of intermolecular polar head group interactions of phospholipids in bilayered vesicles. In the case of the cobra venom enzyme, removing this ammonium group breaks the interaction of the inhibitor with the enzyme (Fig. 3) and with the bilayer; thus, such a change is expected to produce little, if any, effect on the K_1^* values, as is observed.

E* HAS INTRINSICALLY HIGHER AFFINITY FOR INHIBITORS COMPARED WITH E

The binding of phospholipid analogs containing short hydrocarbon chains to PLA₂ in the aqueous phase can be studied because such compounds are water soluble as solitary monomers below their critical micelle concentrations or solubility limit. The apparent affinity of phosphonate phospholipid analog 1 for cobra venom sPLA₂ in the aqueous phase increases enormously in the presence of the nonhydrolyzable short-chain substrate analog 1,2-dihexyl-*sn*-glycero-3-phosphocholine (85). In this study, the binding of radiolabeled inhibitor 1 to cobra venom sPLA₂ was measured by equilibrium dialysis, and the K_1 for the enzyme-inhibitor complex is about 50 μM . Thus, in the aqueous

phase, compound 1 acts as a rather weak-binding inhibitor. The addition of 1,2-dihexyl-sn-glycero-3-phosphocholine to the water-soluble cobra venom enzyme leads to the formation of a protein/lipid microaggregate that can be detected by gel filtration or analytical ultracentrifugation (86-88). Aggregation is also seen with the pancreatic sPLA₂ in the presence of short-chain anionic amphiphiles (89, 90). These aggregates presumably form by the ability of the interfacial recognition surface of the PLA₂ to recruit short-chain, water-soluble phospholipids in a process that leads to the segregation of such complexes to form a protein-lipid microaggregate. Thus, in the presence of 1,2-dihexyl-sn-glycero-3-phosphocholine, the cobra venom PLA₂ may have the properties more of E* than of E. The fact that the inhibitor binds more tightly to the enzyme in the presence of this microaggregation suggests that E* has intrinsically higher affinity for E* than does E.

These results are only qualitative, and they have sparked the design of additional and more rigorous experiments that address the intrinsic affinity of E vs. E* for active site ligands (84). One problem that immediately arises is that it is not possible to directly compare the values of K_i (for the process EI <→ E + I) to K_{i*} (for the process E*I <→ E* + I*), as the former has units of molarity, and the latter has units of mole fraction. Instead it is most useful to consider these equilibrium processes in terms of Scheme II shown below.



Scheme II

In this scheme all the underlying equilibria are considered for the interaction of the inhibitor with the enzyme both in the aqueous phase and at the interface. Thus, in addition to the dissociation constants K_i and K_{i*} discussed previously, the constants K_d and K_d' are the equilibrium constants for the dissociation of E* and E*I, respectively, from the interface of the neutral diluent into the aqueous phase. Thus, K_d = [neutral diluent][E]/[E*] and K_d' = [neutral diluent][E]/[E*I] where quantities in brackets are in units of moles per unit volume. The remaining constant, K', is the equilibrium constant for the dissociation of the inhibitor from the interface into the aqueous phase (K' = [neutral diluent][I]/[I*]).

Equation 2 is a thermodynamic relation (detailed-balance condition) derived from Scheme II.

$$K_d/K_d' = K_i/(K_i \cdot K') = [E][E^*]/[E^*][EI] \quad (2)$$

According to this equation, if K_d/K_d' > 1, [E*I]/[E*] > [EI]/[E], and thus regardless of the total amount of inhibitor in the system, the fraction of enzyme in the interface that is bound to inhibitor is higher than that for the enzyme in the

aqueous phase. Using the thermodynamic cycle (Scheme II) in this way provides a direct evaluation of the intrinsic inhibitor affinity and automatically accounts for differences in local concentrations of inhibitor in the aqueous phase and at the interface.

For all true competitive inhibitors of the pig pancreatic sPLA₂ studied, it is found that K_d/K_d' is approximately 50 (84). This strengthening of the enzyme-interface binding could come about in three different ways: 1) It could be due to a residual inhibitor-interface interaction where part of the inhibitor remains attached to the interface even when the inhibitor has entered the catalytic site; 2) The interface could somehow be influenced by the inhibitor moving into the catalytic site so that it makes better contact with the enzyme; 3) The enzyme may undergo a conformation change when the inhibitor binds so that it makes better contact with the interface. The first two possibilities would suggest that the length of the alkyl chains of the inhibitors would influence the strength of the effect; surprisingly, this is not the case (84). Thus, the most likely effect is an allosteric modulation of the enzyme that occurs when the inhibitor binds in the catalytic site.

PLA₂ INHIBITION STUDIES IN LIVING CELLS

Now that some PLA₂s in cells have been identified and true competitive inhibitors that are enzyme selective have been developed, it should be possible to take a pharmacological approach toward determining the roles that these enzymes play in biological processes. Of course, such an approach does not provide unequivocal data because unidentified PLA₂s may exist in cells, and the full spectrum of enzyme selectivity of any given inhibitor is not known *a priori*.

The presence of AACOCF₃ at concentrations of 0-20 μM blocks essentially all the arachidonic acid that is liberated in thrombin-stimulated platelets (91, 92), in calcium ionophore-stimulated human monocytic cells (U937) (92), and in interleukin-1α-stimulated mesangial cells (93). However, such effects are not seen when the cells are treated with analogs of AACOCF₃, which have minor structural alterations that obliterate their abilities to inhibit cPLA₂ (see above). Such results argue strongly for the role of the cPLA₂ in liberating arachidonate for the biosynthesis of eicosanoids. Furthermore, they allay concerns about using membrane-residing inhibitors to inhibit the action of cPLA₂ in membranes.

Phospholipid analog 2 protects isolated rat lungs from ischemia reperfusion injury when it is delivered as a suspension in liposomes, whereas micelles of this compound added directly to the perfusion medium are ineffective (36). Compound 2 also inhibits the degradation of surfactant phosphatidylcholine by rat lung (94). Biochemical studies suggest that the primary inhibitory effect of this compound is on a calcium-independent PLA₂ of lysosomal origin with optimum activity at acidic pH. This activity is probably involved in the turnover of phospholipids of lung surfactant. This enzyme not only hydrolyzes normal phospholipids but is also responsible for the selective release of conjugated dienes and thiobarbituric acid reactive substances; however, the phospholipid cleavage leading to the formation of thromboxane is not affected.

A substituted bromoenoyl lactone is a specific inhibitor of the calcium-independent PLA₂ from myocardium, and this compound blocks arachidonate liberation in hormonally stimulated rat smooth muscle cells (51).

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REFERENCES

- Waite, M. (1987) *The phospholipases*. Plenum, New York.
- Verheij, H. M., Slotboom, A. J., and de Haas, G. H. (1981) Pancreatic phospholipase A₂: a model for membrane-bound enzymes? *Rev. Physiol. Biochem. Pharmacol.* **91**, 91-203.
- Dennis, E. A. (1983) Phospholipases. *The Enzymes* **16**, 307-353.
- Kramer, R. M., Jakubowski, J. A., and Deykin, D. (1988) Hydrolysis of 1-alkyl-2-arachidonoyl-sn-glycerol-3-phosphocholine, a common precursor of platelet-activating factor and eicosanoids, by human platelet phospholipase A₂. *Biochim. Biophys. Acta* **959**, 269-279.
- Seilhamer, J. J., Pruzanski, W., Vadas, P., Plant, S., Miller, J. A., Kloss, J., and Johnson, L. K. (1989) Cloning and recombinant expression of phospholipase A₂ present in rheumatoid arthritic synovial fluid. *J. Biol. Chem.* **264**, 5335-5338.
- Yu, B.-Z., Berg, O. G., and Jain, M. K. (1993) The divalent cation is obligatory for the binding of ligands to the catalytic site of secretory phospholipase A₂. *Biochemistry* **32**, 6485-6492.
- van den Bergh, C. J., Slotboom, A. J., Verheij, H. M., and de Haas, G. H. (1989) The role of Asp-19 and other conserved amino acids in phospholipase A₂ and their importance for enzymatic activity. *J. Cell. Biochem.* **39**, 379-390.
- Scott, D. L., White, S. P., Otwowski, Z., Yuan, W., Gelb, M. H., and Sigler, P. B. (1990) Interfacial catalysis: the mechanism of phospholipase A₂. *Science* **250**, 1541-1546.
- Wery, J.-P., Schevitz, R. W., Clawson, D. K., Bobbitt, J. L., Dow, E. R., Gamboa, G. T., Goodson, J., Hermann, R. B., Kramer, R. M., McClure, D. B., Mihelich, E. D., Putnam, J., Sharp, J. D., Stark, D. H., Teater, C., Warrick, M. W., and Jones, N. D. (1991) Structure of recombinant human rheumatoid arthritic synovial fluid phospholipase A₂ at 2.2 angstroms resolution. *Nature (London)* **352**, 79-82.
- Thunissen, M. M. G. M., Ab, E., Kalk, K. H., Drent, J., Dijkstra, B. W., Kuipers, O. P., Dijkman, R., de Haas, G. H., and Verheij, H. M. (1990) X-ray structure of phospholipase A₂ complexed with a substrate-derived inhibitor. *Nature (London)* **347**, 689-691.
- Fremont, D. H., Anderson, D. H., Wilson, I. A., Dennis, E. A., and Xuong, N. H. (1993) Crystal structure of phospholipase A₂ from Indian cobra reveals a trimeric association. *Proc. Natl. Acad. Sci. USA* **90**, 342-346.
- Holland, D. R., Clancy, L. L., Muchmore, S. W., Ryde, T. J., Einspahr, H. M., Finzel, B. C., Heinrikson, R. L., and Waterpauw, K. D. (1990) The crystal structure of a lysine-49 phospholipase A₂ from the venom of the cottonmouth snake at 2.0-A resolution. *J. Biol. Chem.* **265**, 17649-17656.
- Westerlund, B., Nordlund, P., Uhlin, U., Eaker, D., and Ekstrand, H. (1992) The three-dimensional structure of notexin, a presynaptic neurotoxic phospholipase A₂ at 2.0 angstrom resolution. *FEBS Lett.* **301**, 159-164.
- Tomoo, K., Ohishi, H., Doi, M., Ishida, T., Inoue, M., Ikeda, K., Hata, Y., and Samejima, Y. (1992) Structure of the acidic phospholipase A₂ for the venom of *Aktitesodon hatus blomhoffi* at 2.8 angstroms resolution. *Biochem. Biophys. Res. Commun.* **184**, 137-143.
- Scott, D. L., White, S. P., Browning, J. L., Rosa, J. J., and Gelb, M. H. (1991) Structures of free and inhibited human secretory phospholipase A₂ from inflammatory exudate. *Science* **254**, 1007-1010.
- Ghomashchi, F., Yu, B.-Z., Berg, O., Jain, M. K., and Gelb, M. H. (1991) Interfacial catalysis by phospholipase A₂: substrate specificity in vesicles. *Biochemistry* **30**, 7318-7329.
- Kim, D. K., Kudo, I., and Inoue, K. (1991) Purification and characterization of rabbit platelet cytosolic phospholipase A₂. *Biochim. Biophys. Acta* **1083**, 80-88.
- Takayama, K., Kudo, I., Kim, D. K., Nagata, K., Nozawa, Y., and Inoue, K. (1991) Purification and characterization of human platelet phospholipase A₂ which preferentially hydrolyses an arachidonyl residue. *FEBS Lett.* **282**, 326-330.
- Kim, D. K., Suh, P. G., and Ryu, S. H. (1991) Purification and some properties of a phospholipase A₂ from bovine platelets. *Biochem. Biophys. Res. Commun.* **174**, 189-196.
- Leslie, C. C., Voelker, D. R., Channon, J. Y., Wall, M. M., and Zelarney, P. T. (1988) Properties and purification of an arachidonyl-hydrolyzing phospholipase A₂ from a macrophage cell line RAW 264.7. *Biochim. Biophys. Acta* **963**, 476-492.
- Wijkander, J., and Sundler, R. (1989) A phospholipase A₂ hydrolyzing arachidonyl-phospholipids in mouse peritoneal macrophages. *FEBS Lett.* **244**, 51-54.
- Wijkander, J., and Sundler, R. (1991) An 100-kDa arachidonate-mobilizing PLA₂ in mouse spleen and the macrophage cell line J774. *Eur. J. Biochem.* **22**, 873-880.
- Clark, J. D., Lin, L.-L., Kriz, R. W., Ramesha, C. S., Sultzman, L. A., Lin, A. Y., Milona, N., and Knopf, J. L. (1991) A novel arachidonic acid-selective cytosolic PLA₂ contains a Ca²⁺-dependent translocation domain with homology to PKC and GAP. *Cell* **65**, 1043-1051.
- Diez, E., and Mong, S. (1990) Purification of a phospholipase A₂ from human monocytic leukemic U-937 cells. *J. Biol. Chem.* **265**, 14654-14661.
- Kramer, R. M., Roberts, E. F., Manetta, J., and Putnam, J. E. (1991) The Ca²⁺-sensitive cytosolic phospholipase A₂ is a 100-kDa protein in human monoblast U-937 cells. *J. Biol. Chem.* **266**, 5268-5272.
- Rehfeldt, W., Hass, R., and Goppelt-Strube, M. (1991) Characterization of phospholipase A₂ in monocytic cell lines. *Biochem. J.* **276**, 631-636.
- Gronich, J. H., Bonventre, J. V., and Nemenoff, R. A. (1990) Purification of a high-molecular mass form of phospholipase A₂ from rat kidney activated at physiological calcium concentrations. *Biochem. J.* **271**, 37-43.
- Channon, J. Y., and Leslie, C. C. (1990) A calcium-dependent mechanism for association of a soluble arachidonyl-hydrolyzing phospholipase A₂ with membrane in the macrophage cell line RAQ 624.7. *J. Biol. Chem.* **265**, 5409-5413.
- Krause, H., Dieter, P., Schulze-Specking, A., Ballhorn, A., and Decker, K. (1991) Ca²⁺-induced reversible translocation of phospholipase A₂ between the cytosol and the membrane fraction of rat liver macrophages. *Eur. J. Biochem.* **199**, 353-359.
- Yoshihara, Y., and Watanabe, Y. (1990) Translocation of phospholipase A₂ from cytosol to membranes in rat brain induced by calcium ions. *Biochem. Biophys. Res. Commun.* **170**, 484-490.
- Wijkander, J., and Sundler, R. (1992) Macrophage arachidonate-mobilizing phospholipase A₂: role of Ca²⁺ for membrane binding but not for catalytic activity. *Biochem. Biophys. Res. Commun.* **184**, 118-124.
- Ghoshashchi, F., Schüttel, S., Jain, M. K., and Gelb, M. H. (1992) Kinetic analysis of a high-molecular-weight phospholipase A₂ from rat kidney: divalent metal-dependent trapping of enzyme on product-containing vesicles. *Biochemistry* **31**, 3814-3824.
- Sharp, J. D., White, D. L., Chiou, X. G., Goodson, T., Gamboa, G. C., McClure, D., Burgett, S., Hoskins, J., Skatrud, P. L., Sportsman, J. R., Becker, G. W., Kang, L. H., Roberts, E. F., and Kramer, R. M. (1991) Molecular cloning and expression of human Ca²⁺-sensitive cytosolic phospholipase A₂. *J. Biol. Chem.* **266**, 14850-14853.
- Gross, R. W. (1992) Myocardial phospholipases A₂ and their membrane substrates. *Trends Cardiovasc. Med.* **2**, 115-121.
- Hirashima, Y., Farooqui, A. A., Mills, J. S., and Horrocks, L. A. (1992) Identification and purification of a calcium-independent phospholipase A₂ from bovine brain cytosol. *J. Neurochem.* **59**, 708-714.
- al-Mehdi, A. B., Dodia, C., Jain, M. K., and Fisher, A. B. (1993) A phospholipase A₂ inhibitor decreases generation of thiobarbituric acid reactive substance during lung ischemia-reperfusion. *Biochim. Biophys. Acta* **1167**, 56-62.
- Glaser, K. B., Mobilio, D., Chang, J. Y., and Senko, N. (1993) Phospholipase A₂ enzymes: regulation and inhibition. *Trends Pharmacol. Sci.* **14**, 92-98.
- Bonalański, J. S., Lawton, P., and Browning, J. L. (1991) Human extracellular recombinant PLA₂ induces an inflammatory response in rabbit joints. *J. Immunol.* **146**, 3904-3910.
- Elsbach, P., and Weiss, J. (1993) The bactericidal/permeability-increasing protein (BPI): a potent element in host-defense against gram-negative bacteria and lipopolysaccharide. *Immunobiology* **187**, 417-429.
- Mori, H., Hara, S., Mizushima, H., Kudo, I., and Inoue, K. (1991) Extracellular PLA₂ detected at inflamed sites in rats does not originate from platelets. *Agents Actions* **32**, 266-269.
- Vadas, P., and Pruzanski, W. (1993) Induction of group II phospholipase A₂ expression and pathogenesis of the sepsis syndrome. *Crit. Rev. Shock* **39**, 160-167.
- Buchler, M., Delier, A., Malfertheiner, P., Kleine, H. O., Wiedeck, H., Uhl, W., Samter, M., Friess, H., Nevalainen, T., and Beger, H. G. (1989) Serum phospholipase A₂ in intensive care patients with peritonitis, multiple injury, and necrotizing pancreatitis. *Klin. Wochenschr.* **67**, 217-21.
- Murakami, M., Kudo, I., Suwa, Y., and Inoue, K. (1992) Release of 14-kDa group-II phospholipase A₂ from activated mast cells and its possible involvement in the regulation of the degranulation process. *Eur. J. Biochem.* **209**, 257-265.
- Asaoka, Y., Yoshida, K., Sasaki, Y., Nishizuka, Y., Murakami, M., Kudo, I., and Inoue, K. (1993) Possible role of mammalian secretory group II phospholipase A₂ in T-lymphocyte activation: implication in propagation of inflammatory reaction. *Proc. Natl. Acad. Sci. USA* **90**, 716-719.

REVIEWS

45. Murakami, M., Kudo, I., and Inoue, K. (1993) Molecular nature of phospholipases A₂ involved in prostaglandin E₂ synthesis in human umbilical vein endothelial cells. Possible participation of cytosolic and extracellular type II phospholipases A₂. *J. Biol. Chem.* **268**, 839-844.
46. Barbour, S. E., and Dennis, E. A. (1993) Antisense inhibition of group II phospholipase A₂ expression blocks the production of prostaglandin E₂ by P388DI cells. *J. Biol. Chem.* **268**, 21875-21882.
47. Arita, H., and Hanasaki, K. (1993) Physiological aspects of a high affinity binding site for pancreatic-type phospholipase A₂. *J. Lipid Mediat.* **6**, 217-222.
48. Lin, L. L., Wartmann, M., Lin, A. Y., Knopf, J. L., Seth, A., and Davis, R. J. (1993) cPLA₂ is phosphorylated and activated by MAP kinase. *Cell* **72**, 269-278.
49. Qiu, Z.-H., de Carvalho, M. S., and Leslie, C. C. (1993) Regulation of phospholipase A₂ activation by phosphorylation in mouse peritoneal macrophages. *J. Biol. Chem.* **268**, 24506-24513.
50. Kramer, R. M., Roberts, E. F., Manetta, J. V., Sportsman, J. R., and Jakubowski, J. A. (1993) Ca(2+)-sensitive cytosolic phospholipase A₂ (cPLA₂) in human platelets. *J. Lipid Mediat.* **6**, 209-216.
51. Lehman, J. J., Brown, K. A., Ramanadham, S., Turk, J., and Gross, R. W. (1993) Arachidonic acid release from aortic smooth muscle cells induced by [Arg8]vasopressin is largely mediated by calcium-independent phospholipase A₂. *J. Biol. Chem.* **268**, 20713-6.
52. Jain, M. K., Rogers, J., Jahagirdar, D. V., Merecek, J. F., and Ramirez, F. (1986) Kinetics of interfacial catalysis by phospholipase A₂ in intravesicle scooting mode, and heterofusion of anionic and zwitterionic vesicles. *Biochim. Biophys. Acta* **860**, 435-447.
53. Jain, M. K., Ranadive, G., Yu, B.-Z., and Verheij, H. M. (1991) Interfacial catalysis by phospholipase A₂: monomeric enzyme is fully catalytically active at the bilayer interface. *Biochemistry* **30**, 7330-7340.
54. Bayburt, T., Yu, B. Z., Lin, H. K., Browning, J., Jain, M. K., and Gelb, M. H. (1993) Human nonpancreatic secreted phospholipase A₂: interfacial parameters, substrate specificities, and competitive inhibitors. *Biochemistry* **32**, 573-582.
55. Hanel, A. M., Schuttel, S., and Gelb, M. H. (1993) Processive interfacial catalysis by mammalian 85-kilodalton phospholipase A₂ enzymes on product-containing vesicles: application to the determination of substrate preferences. *Biochemistry* **32**, 5949-5958.
56. Jain, M. K., Egmond, M. R., Verheij, H. M., Apitz-Castro, R., Dijkman, R., and de Haas, G. H. (1982) Interaction of phospholipase A₂ and phospholipid bilayers. *Biochim. Biophys. Acta* **688**, 341-348.
57. Jain, M. K., and Berg, O. (1989) The kinetics of interfacial catalysis by phospholipase A₂ and regulation of interfacial activation: hopping versus scooting. *Biochim. Biophys. Acta* **1002**, 127-156.
58. Roberts, M. F., Deems, R. A., and Dennis, E. A. (1977) Dual role of interfacial phospholipid in phospholipase A₂ catalysis. *Proc. Natl. Acad. Sci. USA* **74**, 1950-1954.
59. Volwerk, J. J., Pietersen, W. A., and de Haas, G. H. (1974) Histidine at the active site of phospholipase A₂. *Biochemistry* **13**, 1446-1454.
60. Ramirez, F., and Jain, M. K. (1991) Phospholipase A₂ at the bilayer interface. *Proteins* **9**, 229-239.
61. Apitz-Castro, R., Jain, M. K., and de Haas, G. H. (1982) Origin of the latency phase during the action of phospholipase A₂ on unmodified phosphatidylcholine vesicles. *Biochim. Biophys. Acta* **688**, 349-356.
62. Bell, J. D., and Biltonen, R. L. (1992) Molecular details of the activation of soluble phospholipase A₂ on lipid bilayers. Comparison of computer simulations with experimental results. *J. Biol. Chem.* **267**, 11046-11056.
63. Jain, M. K., Gelb, M. H., Rogers, J., and Berg, O. G. (1994) The kinetic basis for interfacial catalysis by phospholipase A₂. *Methods Enzymol.* In press.
64. Gelb, M. H., Jain, M. K., and Berg, O. (1992) Interfacial enzymology of phospholipase A₂. *Bioorg. Med. Chem. Lett.* **2**, 1335-1342.
65. Jain, M. K., Yu, B.-Z., Rogers, J., Ranadive, G. N., and Berg, O. (1991) Interfacial catalysis by phospholipase A₂: dissociation constants for calcium, substrate, and competitive inhibitors. *Biochemistry* **30**, 7306-7317.
66. Jain, M. K., Yuan, W., and Gelb, M. H. (1989) Competitive inhibition of phospholipase A₂ in vesicles. *Biochemistry* **28**, 4135-4139.
67. Jain, M. K., and Jahagirdar, D. V. (1985) Action of phospholipase A₂ on bilayers. Effect of inhibitors. *Biochim. Biophys. Acta* **814**, 319-326.
68. Jain, M. K., Ghomaschi, F., Yu, B.-Z., Bayburt, T., Murphy, D., Houck, D., Brownell, J., Reid, J. C., Solowiej, J. E., Wong, S.-M., Merecek, U., Jarrell, R., Sasser, M., and Gelb, M. H. (1992) Fatty acid amides: scooting mode-based discovery of tight-binding competitive inhibitors of secreted phospholipases A₂. *J. Med. Chem.* **35**, 3584-3586.
69. Jain, M. K., Yu, B.-Z., Gelb, M. H., and Berg, O. G. (1992) Assay of phospholipase A₂ and their inhibitors by kinetic analysis in the scooting mode. *Med. Inflamm.* **1**, 85-100.
70. Ransac, S., Riviere, C., Soulie, J. M., Gancet, C., Verger, R., and de Haas, G. H. (1990) Competitive inhibition of lipolytic enzymes. *Biochim. Biophys. Acta* **1043**, 57-66.
71. Yu, L., and Dennis, E. A. (1991) Thio-based phospholipase assay. *Methods Enzymol.* **197**, 65-75.
72. Yu, L., and Dennis, E. A. (1991) Critical role of a hydrogen bond in the interaction of phospholipase A₂ with transition-state and substrate analogues. *Proc. Natl. Acad. Sci. USA* **88**, 9325-9329.
73. Street, I. P., Lin, H. K., Laliberte, F., Ghomaschi, F., Wang, Z., Perrier, H., Tremblay, N. M., Huang, Z., Weech, P. K., and Gelb, M. H. (1993) Slow- and tight-binding inhibitors of the 85-kDa human phospholipase A₂. *Biochemistry* **32**, 5935-5940.
74. Jain, M. K., Rogers, J., Hendrickson, H. S., and Berg, O. G. (1991) The chemical step is not rate-limiting during the hydrolysis by phospholipase A₂ of mixed micelles of phospholipid and detergent. *Biochemistry* **32**, 8360-8367.
75. Jain, M. K., Rogers, J., Berg, O., and Gelb, M. H. (1991) Interfacial catalysis by phospholipase A₂: activation by substrate replenishment. *Biochemistry* **30**, 7340-7348.
76. Dijkman, R., Lugtigheid, R. B., Dekker, N., Van, D. B. L., Egmond, M. R., and Verheij, H. M. (1993) Competitive inhibition of lipolytic enzymes. IX. A comparative study on the inhibition of pancreatic phospholipases A₂ from different sources by (R)-2-acylamino phospholipid analogues. *Biochim. Biophys. Acta* **1167**, 281-288.
77. Yuan, W., and Gelb, M. H. (1988) Phosphonate-containing phospholipid analogues as tight-binding inhibitors of PLA₂. *J. Am. Chem. Soc.* **110**, 2665-2666.
78. Jain, M. K., Yuan, W., and Gelb, M. H. (1989) Competitive inhibition of phospholipase A₂ in vesicles. *Biochemistry* **28**, 4135-4139.
79. Jain, M. K., Tao, W., Rogers, J., Arendon, C., Eibl, H., and Yu, B.-Z. (1991) Active-site specific competitive inhibitors of PLA₂: novel transition state analogs. *Biochemistry* **30**, 10256-10268.
80. Plesniak, L. A., Boegeman, S. C., Segelke, B. W., and Dennis, E. A. (1993) Interaction of phospholipase A₂ with thioether amide containing phospholipid analogues. *Biochemistry* **32**, 5009-5016.
81. Trimble, L. A., Street, I. P., Perrier, H., Tremblay, N. M., Weech, P. K., and Bernstein, M. A. (1993) NMR structural studies of the tight complex between a trifluoromethyl ketone inhibitor and the 85-kDa human phospholipase A₂. *Biochemistry* **32**, 12360-12365.
82. Morgan, B. P., Scholtz, J. M., Ballinger, M. D., Zipkin, I. D., and Bartlett, P. A. (1991) Differential binding energy: a detailed evaluation of the influence of hydrogen-bonding and hydrophobic groups on the inhibition of thermolysin by phosphorous-containing inhibitors. *J. Am. Chem. Soc.* **113**, 297-307.
83. Lin, H.-K., and Gelb, M. H. (1993) Competitive inhibition of interfacial catalysis by phospholipase A₂: differential interaction of inhibitors with the vesicle interface as a controlling factor of inhibitor potency. *J. Am. Chem. Soc.* **115**, 3932-3942.
84. Jain, M. K., Yu, B.-Z., and Berg, O. G. (1993) Relationship of interfacial equilibria to interfacial activation of phospholipase A₂. *Biochemistry* **32**, 11319-11329.
85. Yuan, W., Quinn, D. M., Sigler, P. B., and Gelb, M. H. (1990) Kinetic and inhibition studies of phospholipase A₂ with short-chain substrates and inhibitors. *Biochemistry* **29**, 6082-6094.
86. Bukowski, T., and Teller, D. C. (1986) Self-association and active enzyme forms of *Naja naja naja* and *Crotalus atrox* phospholipases A₂ studied by analytical ultracentrifugation. *Biochemistry* **25**, 8024-8033.
87. Pluckthun, A., and Dennis, E. A. (1985) Activation, aggregation, and product-inhibition of cobra venom phospholipase A₂ and comparison with other phospholipases. *J. Biol. Chem.* **260**, 11099-11106.
88. van Eijk, J. H., Verheij, H. M., Dijkman, R., and de Haas, G. H. (1983) Interaction of phospholipase A₂ from *Naja melanoleuca* snake venom with monomeric substrate analogs: Activation of the enzyme by protein-protein or lipid-protein interactions. *Eur. J. Biochem.* **132**, 183-188.
89. van Oort, M. G., Dijkman, R., Hille, J. D., and de Haas, G. H. (1985) Kinetic behavior of porcine pancreatic phospholipase A₂ on zwitterionic and negatively charged double-chain substrates. *Biochemistry* **24**, 7993-7999.
90. Rogers, J., Yu, B.-Z., and Jain, M. K. (1992) Basis for the anomalous effect of competitive inhibitors on the kinetics of hydrolysis of short-chain phosphatidylcholines by phospholipase A₂. *Biochemistry* **31**, 6056-6062.
91. Bartoli, F., Lin, H.-K., Ghomaschi, F., Gelb, M. H., Jain, M. K., and Apitz-Castro, R. (1994) Tight-binding inhibitors of 85 kDa phospholipase A₂ but not 14 kDa phospholipase A₂ inhibit the release of arachidonate in thrombin-stimulated human platelets. *J. Biol. Chem.* **269**, 15625-15630.
92. Riendeau, D., Guay, J., Weech, P. K., Laliberte, F., Vergey, J., Li, C., Desmarais, S., Perrier, H., Liu, S., Nicoll-Griffith, D., and Street, I. P. (1994) Arachidonyl trifluoromethyl ketone, a potent inhibitor of 85-kDa phospholipase A₂, blocks production of arachidonate and 12-hydroxyeicosatetraenoic acid by calcium ionophore-challenged platelets. *J. Biol. Chem.* **269**, 1-6.
93. Gronich, J., Konieczkowski, M., Gelb, M. H., Nemenoff, R. A., and Sedor, J. R. (1994) Interleukin 1 α causes rapid activation of cytosolic phospholipase A₂ by phosphorylation in rat mesangial cells. *J. Clin. Invest.* **93**, 1224-1233.
94. Fisher, A. B., Dodia, C., Chander, A., and Jain, M. (1992) A competitive inhibitor of phospholipase A₂ decreases surfactant phosphatidylcholine degradation by the rat lung. *Biochem. J.* **288**, 407-411.

Periodicity of leucine and tandem repetition of a 24-amino acid segment in the primary structure of leucine-rich α_2 -glycoprotein of human serum

(protein structure/amphiphilic structure/gene duplication/membrane protein/galactosamine and glucosamine oligosaccharides)

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ABSTRACT The complete primary structure of the 3.1S leucine-rich α_2 -glycoprotein (LRG) present in human plasma has been determined. This protein ($M_r \approx 45,000$) consists of a single polypeptide chain with one galactosamine and four glucosamine oligosaccharides attached. The polypeptide has two intrachain disulfide bonds and contains 312 amino acid residues of which 66 are leucine. The amino acid sequence can be exactly divided into 13 segments of 24 residues each, eight of which exhibit a periodic pattern in the occurrence of leucine, proline, and asparagine. The consensus sequence for the repeating tetracosapeptide unit is Pro-Xaa-Xaa-Leu-Leu-Xaa-Xaa-Xaa-Xaa-Xaa-Leu-Xaa-Xaa-Leu-Xaa-Xaa-Asn-Xaa-Leu-Xaa-Xaa-Leu. This periodicity suggests that the unique structure of LRG arose from a series of unequal crossovers of a precursor oligonucleotide sequence that encoded a building block rich in leucine. Overall, the amino acid sequence of LRG is not significantly homologous to the continuous sequence of any protein in the current data base. However, the consensus tetracosapeptide sequence shows strong homology to segments of many mitochondrial proteins, viral envelope proteins, and oncogene proteins that have a high leucine content and transmembrane domains. Tandem repetition of similar segments also occurs in apolipoproteins that have amphipathic helical potential. Prediction of the secondary structure by the Chou-Fasman rules and calculation of the hydrophilic/hydrophobic profile by several methods confirm the tandem repetition of largely hydrophobic structural units; these begin with a β -turn that leads into an organized structure with α -helical or β -sheet potential. These structural characteristics and the homology to mitochondrial proteins and apolipoproteins suggest that LRG is a membrane-derived or membrane-associated protein containing a series of domains capable of bipolar surface orientation.

Although complete amino acid sequences have been reported for about half of the nearly 100 proteins that have been isolated from human plasma (1), little is known about the structure of a series of well-characterized α and β -glycoproteins of uncertain or unknown function (2). We have begun study of some of these as part of a program for investigation of the structure and function of human plasma proteins (3, 4). We report here the complete amino acid sequence of a trace component of unknown function that was isolated from human serum in 1977 by Haupt and Baudner (5). They called it 3.1S leucine-rich α_2 -glycoprotein (LRG) because of its sedimentation coefficient, its unusually high content of leucine, and its electrophoretic mobility. The protein was reported to consist of a single polypeptide chain ($M_r \approx 50,000$) and to contain about 23% carbohydrate by weight (5). To our

knowledge, no other structural data have since been published.

LRG is a trace protein of human plasma. The average content of LRG in adult serum is 2.1 mg/100 ml (5). Although neither the site of synthesis nor the function are known and no relationship to disease has been reported, LRG is of interest because of its unusually high content of leucine, which is about 17% by weight. Thus, about every fifth residue is leucine. Whether the leucine is randomly distributed or occurs in a regular structural pattern could only be determined by amino acid sequencing, which we undertook and completed. We found that the sequence exhibits a novel periodic pattern unlike that previously reported for soluble globular proteins except apolipoproteins (6-8). The single polypeptide chain is composed of 312 amino acid residues, and the sequence can be exactly divided into 13 blocks of 24 residues; eight of these tetraacosapeptide sequences exhibit a periodic pattern in the distribution of leucine and certain other residues. The periodic pattern is highly significant statistically; the segment comparison score in SD units for the top 100 scores is 18.06. This suggests that LRG arose by a series of unequal crossovers of an oligonucleotide sequence that encoded a prototype leucine-rich building block of about 24 amino acid residues.

The periodicity in the primary structure of LRG is due to a recurring pattern of leucine residues that produces a segment similar in hydrophobicity and length to that found in the transmembrane segments of amphiphilic (amphipathic) peptides and proteins (9, 10). Thus, we suggest that LRG may be a membrane-associated or membrane-derived protein. This hypothesis is supported by predictions of the secondary structure and the hydrophilic/hydrophobic character (hydropathy profile) of the polypeptide chain.

MATERIALS AND METHODS

Materials. Purified LRG prepared from human serum by the method of Haupt and Baudner (5) and antiserum specific for LRG were obtained from Behringwerke AG (Marburg/Lahn, F.R.G.). The protein migrated as a single band in Na-DodSO₄/polyacrylamide gel electrophoresis both in the presence and absence of 2-mercaptoethanol.

Methods. Methods used for the determination of the primary structure of proteins in our laboratory have been described (3, 4, 11). The purified LRG was reduced and carboxymethylated before sequence analysis. The carboxymethylated protein (5-15 mg) was subjected to separate digestions with L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin, *Staphylococcus aureus* V8 protease, and endoproteineinase Lys-C. The intact protein was also

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Abbreviation: LRG, leucine-rich α_2 -glycoprotein.

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cleaved with dilute acid at 108°C for 4 hr (12). Each enzymatic digest was separated by a combination of gel filtration and high-performance liquid chromatography (HPLC) on an Ultrasphere ODS column (Altex, Berkeley, CA) or a Synchropak RP-P column (SynChrom, Linden, IN) with a programmed gradient of 1-propanol containing 0.1% trifluoroacetic acid or 0.1% heptafluorobutyric acid (4). The digest with dilute acid was separated by an automated two-step HPLC system; this consists of a combination of anion-exchange chromatography with a Spherogel-TSK IEX-540 DEAE column (Altex) as the first step and chromatography with a reversed-phase Ultrasphere ODS column as the second. The purified peptides were analyzed with the amino acid analyzer, and their sequences were determined by automatic Edman degradation (3, 4, 11). Hexosamine analysis was also done with the amino acid analyzer, after acid hydrolysis (3, 11).

Computer Analysis of Sequence Data. The sequence database of the *Atlas of Protein Sequence and Structure* updated to August 1984 and the programs SEARCH, ALIGN, RELATE, and PRPLOT were provided by the National Biomedical Research Foundation.[†] The programs SEARCH, RELATE, and ALIGN (13) were used either with the unitary matrix or with the mutation data matrix; all gave a score for statistical significance in standard deviations (SD) of the real score above a score of 100 random runs. We used PRPLOT to plot the hydrophilic/hydrophobic profile of the molecule by utilizing different scales including those of Kyte and Doolittle (14), Wolfenden *et al.* (15), Argos *et al.* (16), and Eisenberg (10). PRPLOT was also used to estimate the secondary structure predicted by the procedure of Chou and Fasman (17) based on their values for the tendency of individual amino acids to appear in α -helix, β -sheet, and β -turn structures.

RESULTS AND DISCUSSION

Amino Acid Composition. The amino acid composition calculated from the sequence analysis (Table 1) corresponds well with that obtained by amino acid analysis of the protein (5). The very high content of leucine (66 of 312 residues) is an unusual characteristic of this protein. By amino acid analysis the leucine content of the glycoprotein is 16.62% by weight (5). The value for the polypeptide portion calculated from the complete sequence is 21.75% by weight or 21.12 mole %. A search of the protein sequence data base[†] showed that the leucine content of LRG is exceeded only by those of a vespid venom peptide, a few hypothetical bacteriophage and mitochondrial proteins corresponding to gene sequences, and the hormone secretin. Most other sequences listed that had a high leucine content (15–20 mole %) also were for hypothetical mitochondrial proteins or were for known mitochondrial cytochromes and enzymes, viral coat proteins, or biologically active peptides. About one-third of the amino acid residues of LRG are strongly hydrophobic. Other notable features are the low content of tyrosine (3 residues), isoleucine (4 residues), and cysteine (4 residues). The latter are linked in two disulfide bonds; one is close to the amino terminus and is between cysteine-8 and cysteine-21, and the other is near the carboxyl terminus and is between cysteine-268 and cysteine-294.

Polypeptide Structure and Molecular Weight. Human LRG consists of a single polypeptide chain containing 312 amino acid residues, one GalN oligosaccharide, and four GlcN oli-

Table 1. Amino acid composition of human LRG based on the complete amino acid sequence determination

Amino acid	No. of residues	Amino acid	No. of residues
Aspartic acid	19	Valine	14
Asparagine	17	Methionine	3
Threonine	12	Isoleucine	4
Serine	21	Leucine	66
Glutamic acid	14	Tyrosine	3
Glutamine	20	Phenylalanine	10
Proline	22	Lysine	12
Glycine	23	Histidine	7
Alanine	21	Arginine	15
Half-cystine	4	Tryptophan	5

M, of unmodified polypeptide chain is 34,346; number of residues is 312. Thr-2 is linked to a GalN oligosaccharide. Asn-44, Asn-151, Asn-234, and Asn-290 are linked to GlcN oligosaccharides.

gosaccharides (Fig. 1). The *M*, calculated for the unglycosylated polypeptide chain is 34,346. An *M*, of 35,970 was obtained from electrophoresis in NaDODSO₄/polyacrylamide gels when the results estimated in gels ranging from 5% to 20% gel concentration were extrapolated by use of a double-reciprocal plot. This value, which presumably reflects only the polypeptide chain, is much less than the *M*, of 49,600 \pm 4,000 determined by equilibrium sedimentation (5). However, the carbohydrate content of LRG (about 23% by weight) contributes significantly to the *M*, and also makes it difficult to determine the *M*, accurately by physicochemical methods. We estimate that the *M*, of LRG is approximately 45,000, based on the value of 34,346 for the polypeptide portion and an estimate of 600 for the GalN oligosaccharide and of 2500 for each of the four GlcN oligosaccharides.

Number and Location of Oligosaccharides. Fig. 1 gives the complete amino acid sequence of LRG and shows the sites of attachment of the five oligosaccharides. The GalN is O-linked to the second residue (Thr-2). Both in its proximity to the amino terminus and to a nearby proline, this linkage is similar to that of the GalN attached to the amino-terminal residue (threonine) in human hemopexin (11, 18). However, no obligatory signal sequence for the attachment of GalN is known, whereas GlcN is always N-linked to asparagine in the tripeptide sequence Asn-Xaa-(Thr/Ser). In LRG there are four GlcN oligosaccharides; these are linked to Asn-44, Asn-151, Asn-234, and Asn-290. However, the asparagine is not glycosylated in the putative acceptor sequence Asn-Leu-Ser at positions 271–273. Nothing has been published about the carbohydrate structure of the oligosaccharides of LRG; however, a complex dibranched GlcN oligosaccharide is the most common carbohydrate in human plasma glycoproteins (19).

Internal Homology. Although many plasma proteins exhibit strong evidence of internal duplication in amino acid sequence (20, 21), at first appearance LRG does not. Despite the frequency of leucine residues, there are only five instances of a Leu-Leu sequence and only one case of the triplet sequence Leu-Leu-Leu, compared to 53 instances of single leucine residues. This lack of a statistical distribution puzzled us. However, first by visual inspection and then by computer analysis with the program RELATE, we found that the amino acid sequence of LRG fits a 13-cycle periodic pattern dominated by a 24-residue peptide (tetracosapeptide) which has the consensus sequence Pro-Xaa-Xaa-Leu-Leu-Xaa-Xaa-Xaa-Xaa-Xaa-Leu-Xaa-Xaa-Leu-Xaa-Leu-Xaa-Xaa-Asn-Xaa-Leu-Xaa-Xaa-Leu. All of the amino acid residues identified in this sequence occur at least six times in the alignment shown in Fig. 2. In the computer listing of the 100 segments having the highest scores for intrasequence homology (the top 100 of 39,903 comparisons), the lengths of all

[†]Barker, W. C., Hunt, L. T., Orcutt, B. C., George, D. G., Yeh, L. S., Chen, H. R., Blomquist, M. C., Johnson, G. C. & Dayhoff, M. O. (Aug. 1, 1984) *Atlas of Protein Sequence and Structure, Protein Sequence Database*, (Natl. Biomed. Res. Found., Washington, D.C.).

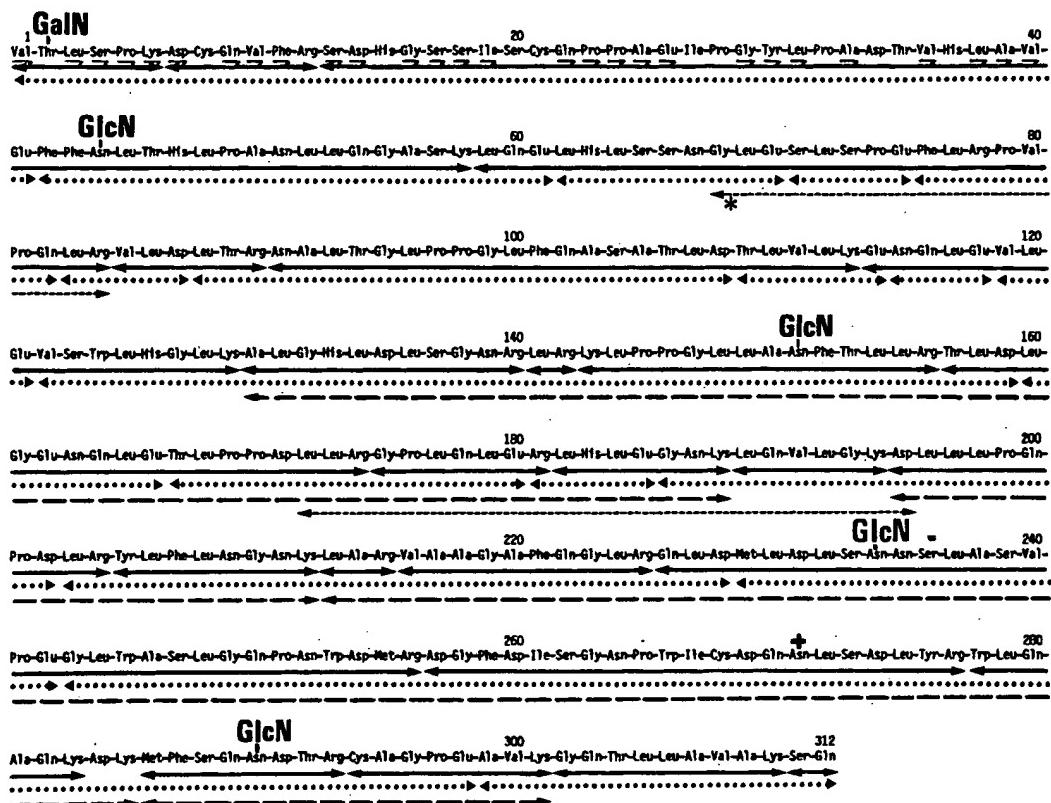


FIG. 1. Summary of the complete amino acid sequence of human LRG. The sequence is shown along with all peptides necessary for the proof of sequence. The peptides obtained from different digestions are as follows: —, tryptic peptides; ······, *S. aureus* V8 peptides; ——, endoproteinase Lys-C peptides; - - - - -, dilute acid peptides. The asterisk indicates a dilute acid peptide of a tryptic peptide. GaN and GlcN indicate the attachment sites for the galactosamine oligosaccharide and the glucosamine oligosaccharides, respectively. A potential glycosylation site (Asn-271) is shown by a plus sign. No evidence for polymorphism in the sequence of LRG was found except for a possible ambiguity at position 215, where tyrosine may replace arginine in some molecules.

segments listed were divisible by 24, and all the segments fit exactly into the periodic pattern of Fig. 2. In this pattern, the complete sequence of 312 residues is exactly divided into an array of 13 tetracosapeptide segments with no gaps inserted. The segment comparison score for the tetracosapeptide segments is 18.06 SD units. A score of 3.0 SD units is considered significant (13).

The unique feature of the 13-cycle alignment is that it is

based mainly on a periodic distribution of a single amino acid rather than on a recurrent sequence of several different amino acids. More than 50 of the 66 leucine residues fit the pattern, including almost all the leucines in the 200 successive residues (nos. 38–237) in which the most homologous tetracosapeptide segments are located. Four more leucines are aligned in column 8 of Fig. 2. Since leucine is one of the most hydrophobic amino acids and is usually present in an or-

1	1	V T O L S P K D C Q V F R S D H G S S I S C Q P P	24
2	25	A E I P G Y L P A D T V H A V E F F N * T H P	48
3	49	G A N P Q G A S K H Q E H S S G G E S	72
4	73	S P E F R P V P V Q R V D T R G A A T T G	96
5	97	P P G H T Q A S A T D T V K E Q E V	120
6	121	E V S H P H G L K A G H D S G R R K	144
7	145	P P G H A N * F T L R T G E G E T	168
8	169	P P D H R G P L Q E R H E G Q V	192
9	193	G K D H L P Q P D R Y F N G N K A R V	216
10	217	A A G A F Q G L R Q D M D S N A S S V	240
11	241	E G W A S L G Q P N W D M R D G F D I S G N	264
12	265	R W I C D Q N L S D Y R W L Q A Q K D K M F S	288
13	289	Q N * D T R C A G P E A V K G Q T L L A V A K S Q	312
Consensus		P P G L L Q G L P Q L R X L D L S G N X L E S L	

FIG. 2. Periodicity of leucine residues and tandem repetition of a 24-amino acid segment in the primary structure of LRG. The sequence of Fig. 1 with position numbers is shown in the one-letter code for amino acids (13) and is aligned exactly into 13 segments (numbered in bold type). Amino acids that occur at least six times in a vertical column are shown in bold type and are enclosed in shaded boxes. The consensus sequence is given at the bottom, with the most frequent residues in bold type. Others that occur three or four times are also given in the consensus sequence, and X is used where there is no clear preference. The cysteine (C) residues are circled and the disulfide bonds are represented schematically. An asterisk denotes each asparagine (N) to which a GlcN oligosaccharide is linked, and a solid circle at threonine-2 indicates that a GaN oligosaccharide is attached.

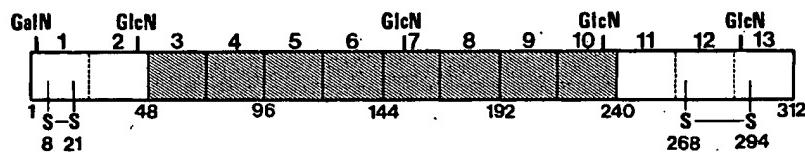


FIG. 3. Schematic representation of the tandem repetition of 24-amino acid segments in LRG. Each numbered block represents a segment (see Fig. 2). Shaded blocks represent homologous segments that exhibit a periodic pattern of leucine and other amino acids. The numbers below the diagram give the sequence positions. The locations of the GalN and GlcN oligosaccharides and of the two disulfide bonds are shown.

dered structure (α -helix or β -pleated sheet), the periodicity of leucine residues probably reflects a unique structural characteristic of LRG that has been attained by a series of unequal crossovers of an oligonucleotide sequence that coded for a prototype building block that was rich in leucine. Although the entire polypeptide sequence is exactly divisible into 13 tetracosapeptide segments, only the central 8 segments exhibit strong inter-segment homology. These comprise a central core structure, illustrated schematically in Fig. 3. The amino-terminal segment has a GalN oligosaccharide and an intra-segment disulfide bond. The periodic pattern of homology ends after segment 10. The last two segments are joined by a disulfide bond, and the last has a GlcN oligosaccharide.

Sequence Homology to Other Proteins. To examine the possible occurrence in other proteins of sequences homologous to the periodic tetracosapeptide of LRG, we used as a probe a consensus sequence that represents the amino acid of highest frequency in each vertical column of Fig. 2. The sequence used was Pro-Pro-Gly-Leu-Leu-Gln-Gly-Leu-Pro-Gln-Leu-Arg-Xaa-Leu-Asp-Leu-Ser-Gly-Asn-Xaa-Leu-Glu-Ser-Leu, in which Xaa indicates that no amino acid occurred with sufficient frequency.

By use of the program SEARCH and both the unitary matrix and the mutation data matrix, we found that LRG has a unique amino acid sequence that does not exhibit significant homology over a series of contiguous segments to any protein in the current sequence data base.[†] However, most of the repeating tetracosapeptide segments of LRG do exhibit striking homology of their basic pattern with segments of a number of polypeptides, most of which have a high leucine content. These polypeptides include many viral envelope proteins, oncogene products, and a number of known mitochondrial proteins, such as various cytochromes as well as hypothetical mitochondrial proteins for which the sequences have been deduced by cloning techniques. For example, when the unitary matrix was used, the highest score for the prototype tetracosapeptide sequence was given by a 24-residue segment of hypothetical protein 4 of the human mitochondrion, which has a leucine content of 21 mole % (22). Other segments of protein 4 and other mitochondrial proteins scored high, but the matching segments were not contiguous in the sequences of the proteins. When the mutation data matrix was used, the highest score was for the hemagglutinin precursor of various strains of human and duck influenza virus (residues 441–464). This segment corresponds to residues 78–101 in the second (HA₂) chain of the processed virus protein and exists as a long α -helix with a 36-Å hydrophobic exterior surface (23).

We do not suggest that the homology identified signifies a genetic or evolutionary relationship. Rather, it probably reflects the fact that a high leucine content may result in a periodicity in sequence similar to that of LRG. In an organized structure such as the α -helix or β -pleated sheet (for both of which leucine has a high potential), the hydrophobic residues tend to cluster in a similar orientation (9, 10). Such hydrophobic patches must either be in the interior of protein molecules or, if on the exterior, will tend to interact with hydrophobic membrane surfaces.

The high solubility of LRG and its resistance to heat coagulation are probably due to the combination of a high carbohydrate content, which greatly increases the hydrophilicity of the protein surface, and to the clustering of the hydrophobic leucine residues, which are probably internalized in the protein.

Tandem Repetitions of Protein Sequences. Although fibrous proteins such as collagen have frequent short (tripeptide) repeating units, tandem repetitions of the length and type found in LRG are rare. However, some apolipoproteins contain a series of 11-amino acid or 22-amino acid segments similar to although not closely homologous with the repeating sequence in LRG (6–8). Fitch (6) identified a 13-cycle repeat of an 11-amino acid (or 22-amino acid) sequence in human apolipoprotein A-I and proposed that it resulted from a series of intragenic unequal crossovers. Barker and Dayhoff (7) identified a similar periodicity in human apolipoproteins A-II, C-II, and C-III and suggested that the four lipoproteins arose from a single ancestral gene. Recently Boguski *et al.* (8) reported that rat apolipoprotein A-IV contains 13 tandem repetitions of a 22-amino acid segment with amphiphatic helical potential. The consensus sequence of the apolipoproteins is similar to that of LRG in length, in the number of repetitions, and in the frequency of leucine and other hydrophobic residues. However, the sequence homology of LRG and apolipoproteins is not pronounced; hence, they probably have a functional rather than a genetic relationship. The apolipoprotein repeating sequence can be drawn as an α -helical wheel with amphiphilic character; that is, the nonpolar residues are segregated on one section of the circumference, and hydrophilic and charged residues are aligned on the rest. The Chou-Fasman rules (17) lead to the prediction of an alternating secondary structure, which is in accord with a plot of the hydropathic index. The result is a helical amphiphilic structure to which the function of lipid binding is attributed.

Hydrophilic/Hydrophobic Profile. When the computer program PRPLOT was used with different scales (10, 14–16) to evaluate the hydrophilic/hydrophobic profile of LRG, the results were in general agreement. Computation of the hydropathic index of Kyte and Doolittle (14) (Fig. 4) shows that the eight conserved segments of the central core are largely hydrophobic, whereas the amino-terminal segment and the last two segments are mainly hydrophilic. Each of the central eight segments has a predominantly hydrophobic pattern with a short hydrophilic section in the middle or near the carboxyl terminus. The patterns for the eight tandem segments are similar but not identical. The carboxyl-terminal segment appears to end with a hydrophobic segment; this could be buried inside the molecule, or it could be a transmembrane segment. In view of the high leucine content, the hydrophobicity of LRG is not unexpected. However, using the method of Eisenberg (10), we were unable to identify a series of potential membrane segments of 21–24 residues in length, such as those he described in some membrane proteins. Also, the periodic leucine residues did not all cluster together in an α -helical wheel as the hydrophobic residues tend to do in apolipoproteins and amphiphilic peptides (8–10).

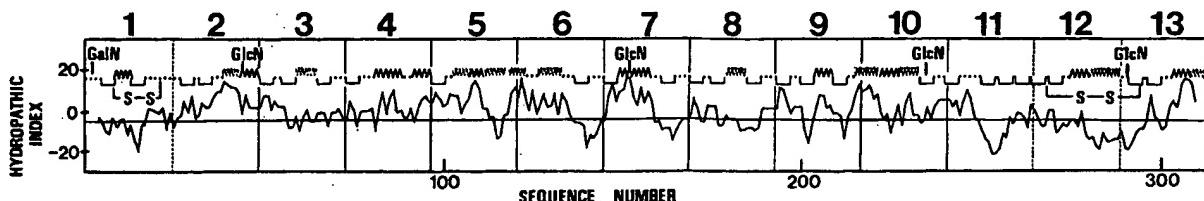


FIG. 4. Hydropathy profile (14) and secondary structure predictions (17) for LRG calculated by use of the PRPLOT program (see text footnote †). In the secondary structure prediction, the residues are represented by dots and are shown in α -helical (www), β -sheet (www), and β -turn ($M\backslash$) conformational states. Locations are given for the oligosaccharides (GlcN and GlcN) and for the disulfide bonds. The vertical lines divide the structure into the 13 numbered tandem segments for which the amino acid sequences are given in Fig. 2.

Secondary Structure. The secondary structure predicted by the Chou-Fasman rules (17) shows some repetition of structural elements in the eight tandem conserved segments of the central core of the molecule (shaded section of Fig. 3). In fact, the predicted structure is nearly identical for segments 3 and 8, which have 11 residues in common. Although the predicted secondary structure of the core segments is not identical, the general pattern is similar. Most of the conserved segments begin with a β -turn, followed by an organized structure (either α -helical or β -sheet) in the middle of the segment, and end with a β -turn. The two segments at the amino terminus and the two at the carboxyl terminus are each the loci of a disulfide bond and of carbohydrate, both of which greatly affect the local structure in a way that is hard to predict by the Chou-Fasman rules. These structural characteristics and the homology to mitochondrial proteins and apolipoproteins suggest that LRG is a membrane-derived or membrane-associated protein containing a series of domains capable of bipolar surface orientation.

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1. Putnam, F. W., ed. (1984) *The Plasma Proteins* (Academic, Orlando, FL), 2nd Ed., Vol. 4.
2. Schwick, H. G. & Haupt, H. (1984) in *The Plasma Proteins*, ed. Putnam, F. W. (Academic, Orlando, FL), 2nd Ed., Vol. 4, pp. 167-220.
3. Lozier, J., Takahashi, N. & Putnam, F. W. (1984) *Proc. Natl. Acad. Sci. USA* 81, 3640-3644.
4. Takahashi, N., Takahashi, Y., Ortel, T. L., Lozier, J., Ishioka, N. & Putnam, F. W. (1984) *J. Chromatogr.* 317, 11-25.
5. Haupt, H. & Baudner, S. (1977) *Hoppe-Seyler's Z. Physiol. Chem.* 358, 639-646.
6. Fitch, W. M. (1977) *Genetics* 86, 623-644.
7. Barker, W. C. & Dayhoff, M. O. (1977) *Comp. Biochem. Physiol. B* 57, 309-315.
8. Boguski, M. S., Elshourbagy, N., Taylor, J. M. & Gordon, J. I. (1984) *Proc. Natl. Acad. Sci. USA* 81, 5021-5025.
9. Kaiser, E. T. & Kézdy, F. J. (1983) *Proc. Natl. Acad. Sci. USA* 80, 1137-1143.
10. Eisenberg, D. (1984) *Annu. Rev. Biochem.* 53, 595-623.
11. Takahashi, N., Takahashi, Y. & Putnam, F. W. (1984) *Proc. Natl. Acad. Sci. USA* 81, 2021-2025.
12. Inglis, A. S. (1983) *Methods Enzymol.* 91, 324-332.
13. Barker, W. C., Ketcham, L. K. & Dayhoff, M. O. (1978) in *Atlas of Protein Sequence and Structure*, Dayhoff, M. O. ed. (Natl. Biomed. Res. Found., Washington, DC), Vol. 5, Suppl. 3, pp. 359-362.
14. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* 157, 105-132.
15. Wolfenden, R., Anderson, L., Cullis, P. M. & Southgate, C. C. B. (1981) *Biochemistry* 20, 849-855.
16. Argos, P., Rao, J. K. M. & Hargrave, P. A. (1982) *Eur. J. Biochem.* 128, 565-575.
17. Chou, P. Y. & Fasman, G. O. (1978) *Annu. Rev. Biochem.* 47, 251-276.
18. Takahashi, N., Takahashi, Y. & Putnam, F. W. (1985) *Proc. Natl. Acad. Sci. USA* 82, 73-77.
19. Baenziger, J. (1984) in *The Plasma Proteins*, ed. Putnam, F. W. (Academic, Orlando, FL), 2nd Ed., Vol. 4, pp. 271-315.
20. Doolittle, R. F. (1984) in *The Plasma Proteins*, ed. Putnam, F. W. (Academic, Orlando, FL), 2nd Ed., Vol. 4, pp. 317-359.
21. Ortel, T. L., Takahashi, N. & Putnam, F. W. (1984) *Proc. Natl. Acad. Sci. USA* 81, 4761-4765.
22. Anderson, S., Bankier, A. T., Barrell, B. G., de Brujin, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R. & Young, I. G. (1981) *Nature (London)* 290, 457-465.
23. Wilson, I. A., Skehel, J. J. & Wiley, D. C. (1981) *Nature (London)* 289, 366-373.

SEQ ID NO: 262

RESULT 8
AAV87784
ID AAV87784 standard; cDNA; 686 BP.
XX
AC AAV87784;
XX
DT 12-FEB-1999 (first entry)
XX
DE EST clone ES35.
XX
KW Expressed sequence tag; secreted protein; haematopoiesis regulator;
KW tissue growth; activin; inhibin; tumour invasion suppressor; EST; human;
KW chemotaxis; chemokinesis; haemostasis; gene therapy; thrombolysis;
KW receptor; ligand; anti-inflammatory; tumour inhibitor; ds.
XX
OS Homo sapiens.
XX
PN WO9845437-A2.
XX
PD 15-OCT-1998.
XX
PF 10-APR-1998; 98WO-US06956.
XX
PR 10-APR-1997; 97US-0837312.
XX
PA (GEMY) GENETICS INST INC.
XX
PI Agostino MJ, Jacobs K, Lavallie ER, McCoy JM, Merberg D;
PI Racie LA, Spaulding V, Treacy M;
XX
DR WPI; 1999-070078/06.
XX
PT New polynucleotides encoding human secreted proteins - derived from
PT e.g. human blood, kidney, foetal lung, placenta, testes, brain,
PT ovary, pituitary, retina and colon cDNA libraries
XX
PS Claim 1; Page 178; 641pp; English.
XX
CC The present sequence represents an expressed sequence tag (EST), and is
CC a polynucleotide of the invention. The polynucleotides of the invention
CC are all secreted EST sequences isolated from a variety of human tissue
CC sources. The EST sequences and proteins encoded by them are predicted to
CC have useful biological activities which would make them suitable for
CC treating, preventing or ameliorating medical conditions in humans and
CC animals, although no supporting data is given. Suggested activities
CC include nutritional activity, immune stimulating or suppressing activity,
CC haematopoiesis regulating activity, tissue growth activity,
CC activin/inhibin activity, chemotactic/chemokinetic activity, haemostatic
CC and thromolytic activity, receptor/ligand activity, anti-inflammatory
CC activity, cadherin/tumour invasion suppressor activity, tumour inhibition
CC activity. The EST sequences are also stated to be useful for gene
CC therapy.
XX
SQ Sequence 686 BP; 137 A; 243 C; 177 G; 129 T; 0 other;

Query Match 32.9%; Score 603; DB 20; Length 686;
Best Local Similarity 99.8%; Pred. No. 3e-267;
Matches 653; Conservative 0; Mismatches 1; Indels 0; Gaps 0;

Qy 69 CTACCATGTCCTCTTGGAGCAGACAGCGACCAAAAAGCCCAGGGGCATTCAACCCATG 128
Db 22 CTACCATGTCCTCTTGGAGCAGACAGCGACCAAAAAGCCCAGGGGCATTCAACCCATG 81

Qy 129 TTTCTAGAACTCTGTTCTGCTGCTGTTGGCAGCCTCAGCCTGGGGGTCAACCCTGA 188
Db 82 TTTCTAGAACTCTGTTCTGCTGCTGTTGGCAGCCTCAGCCTGGGGGTCAACCCTGA 141

Qy 189 GCCCCAAAGACTGCCAGGTGTTCCGCTCAGACCATGGCAGCTCCATCTCCTGTCAACCAC 248
Db 142 GCCCCAAAGACTGCCAGGTGTTCCGCTCAGACCATGGCAGCTCCATCTCCTGTCAACCAC 201

Qy 249 CTGCCGAAATCCCCGGTACCTGCCAGCGACACCGTGACCTGGCGTGGATTCTCA 308
|||
Db 202 CTGCCGAAATCCCCGGTACCTGCCAGCGACACCGTGACCTGGCGTGGATTCTCA 261

Qy 309 ACCTGACCACCTGCCAGCCAACCTCCCTCCAGGGCGCCTCTAACGCTCCAAGAATTGCACC 368
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Db 262 ACCTGACCACCTGCCAGCCAACCTCCCTCCAGGGCGCCTCTAACGCTCCAAGAATTGCACC 321

Qy 369 TCTCCAGCAATGGGCTGGAAAGCCTCTGCCCGAATTCCCTGCCGCAGTGCCGCAGCTGA 428
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Db 502 GGCTACACGGCCTGAAAGCTCTGGGCATCTGGACCTGTCTGGAACCGCCCTCCGAAAC 561

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